PRACTICAL PLANT ANATOMY

AN ELEMENTARY COURSE FOR STUDENTS

BY

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WITH A FOREWORD BY

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PREFACE

THIS book is written with the purpose of assisting students in what usually proves to be the most difficult part of their botanical practical work during the intermediate stage. It is believed that a student who works conscientiously through the course will acquire some appreciable dexterity in using the microscope for studying botanical material and gain a good basic knowledge of the vegetative anatomy of angiosperms.

Much of the subject-matter consists of directions and hints that have been found valuable in personal and teaching experience. The remainder is a set of instructions in relation to exercises the range of which should give sufficient experience to students studying for examinations up to and including the intermediate science of the University of London.

The book is divided for convenience of reference into numbered sections, each of which covers one practical method, a group of closely allied ones or a set of exercises of similar character. Each working method is described just prior to the exercise in which it is first used and reference is made to the description, by the number of the section containing it, in closely following exercises requiring its use. A section number is frequently used in exercise instructions in order to obviate the necessity of repeating a long description of a process.

Three factors have been considered in choosing the sequence of the exercises: difficulty of manipulation of material, difficulty of understanding the prepared object

and the theoretical relationships of the exercises the one to the other. As far as possible the first two have been given most weight, so that difficulty may march hand in hand with increasing dexterity. On the other hand, for the benefit of students who are forced to work with little or no guidance, the difficulty sequence has been broken without hesitation when it has been felt that the theoretical implications of an easier exercise will be best grasped if one of greater difficulty is undertaken first.

Students will find that their progress is at first slow; much has to be learned before a result is attained. Many will feel the impulse to get on somehow and to hurry through those lines of the book which do not seem to lead to the immediate end. Others will at first feel more comfortable working in a way other than what is advised.

Let those who would hurry glance ahead through the book. They will find less and less instructions are given as the course proceeds. More and more knowledge is assumed, and if the preceding pages have been thoroughly assimilated, progress will be faster and faster. No claim is made that the methods suggested in this book are perfect, but they are without doubt ones which give good results and students will be well advised to follow them closely until they are at least moderately skilled. Then perhaps they may modify them to their own taste for the better.

COMYNS J. A. BERKELEY.

CHELSEA POLYTECHNIC,
LONDON, S.W.3.
September 1934.

PREFACE TO SECOND EDITION

TWELVE years' experience with this book has shown that it adequately fulfils its purpose for those students who use it conscientiously. Even though there may not be time for every one of the ninety-seven exercises to be carried out, if the general advice given in somewhat larger type between the exercises is assimilated and a suitable selection of the exercises themselves is completed, sufficient skill usually results. As a consequence, few alterations in the text have seemed necessary. Those that have been made result from suggestions by critical students or from observation of the misapprehensions of the less perspicacious and are made to gain clarity or to give extra emphasis to important points. Thus the section symbol (§) is now used to denote sections of the book, both as a prefix to the numbered section headings and where reference is made to such sections in the text, and the large-scale requisite for high-power drawings is emphasised repeatedly, in places by means of heavy type.

CHELSEA POLYTECHNIC,
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CONTENTS

CHAPTER I

THE COMPOUND MICROSCOPE AND THE METHOD OF USING IT

CHAPTER II

CUTTING AND MOUNTING SIMPLE SECTIONS—
MICRO-CHEMICAL REACTIONS

CHAPTER III

CUTTING, MOUNTING AND DRAWING STEM SECTIONS

CHAPTER IV

OVERCOMING DIFFICULTIES

APPENDIX I

APPENDIX II

APPENDIX III

APPENDIX IV

LIST OF ILLUSTRATIONS

FOREWORD

BOTANY cannot be studied profitably unless the plant itself is investigated, and it is therefore essential that the student shall acquire some facility in a technique which, while presenting no real difficulties, demands care and attention to detail. An attempt has been made in this book to help the student to build up a satisfactory knowledge of botanical technique, by providing a series of exercises and practical hints, all of which have been tested by the author during his considerable experience in teaching students of diverse types. Every exercise is well within the power of anyone of ordinary capacity and application.

The book will be of assistance to students working under guidance, and to those who, for one reason or another, have to find their own way into the delights of practical botany. The student who really masters the exercises will gain, not only a valuable introduction to the study of plant structure, but also that measure of confidence which will help him to direct his own work and to build up his own methods. Those who use this book can best show their appreciation by making it a foundation for a thorough study of the plants.

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CHELSEA POLYTECHNIC,
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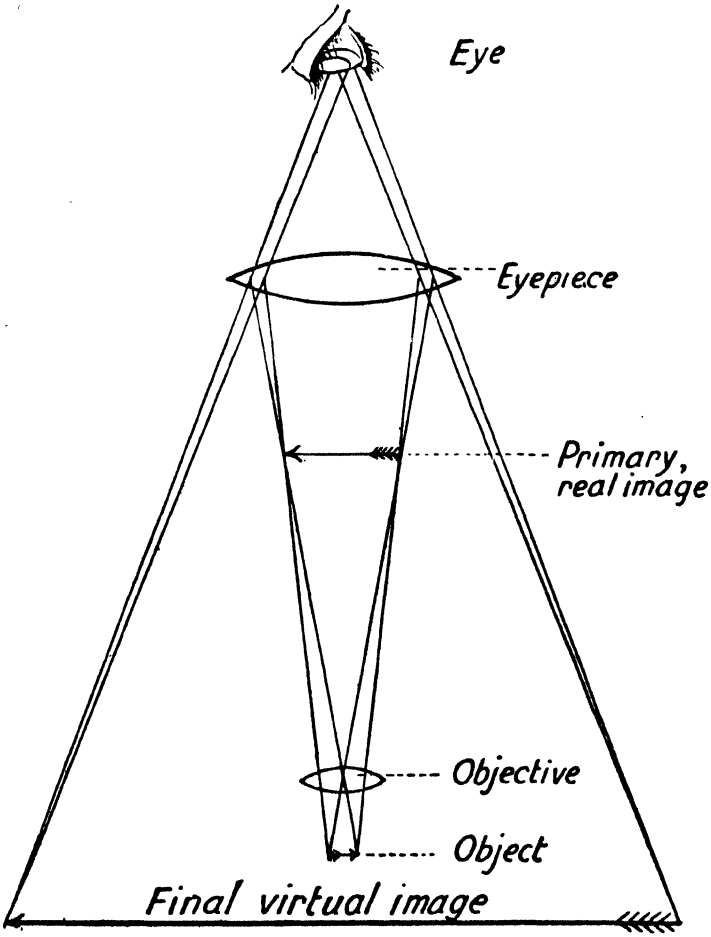

FIG. 1.—Principle of the Compound Microscope.

CHAPTER I

THE COMPOUND MICROSCOPE AND THE METHOD OF USING IT

§ 1. The Principle of the Compound Microscope

WHEN light coming from an object passes through a suitable lens correctly placed (Fig. 1, objective) a primary image larger than the object will be formed on the far side of the lens. This image could be received on a screen and is therefore called a real image. If another suitable lens (eyepiece) is correctly situated between this image and the eye of an observer, another image will appear as if placed farther away than the objective and of much greater size. There is thus formed by the eyepiece a second image which since it cannot be received on a screen is called a virtual image.

The objective forms a real " primary " magnified image of the object and the eyepiece forms a " secondary " and further magnified virtual image of the primary one.

§ 2. The Parts of the Compound Microscope

Fig. 2 indicates the parts of a modern compound microscope which may be considered in four groups.

The Supporting Parts

The **supporting base** or **foot** carries the **arm** or **limb** by means of a transverse joint so that the microscope may be inclined out of the vertical. The arm supports the **body tube** on a rack and pinion and also the stage upon which objects to be examined are placed. The body tube bears

at its lower end the **nosepiece** into which are screwed the objectives. It can be lengthened by means of a draw

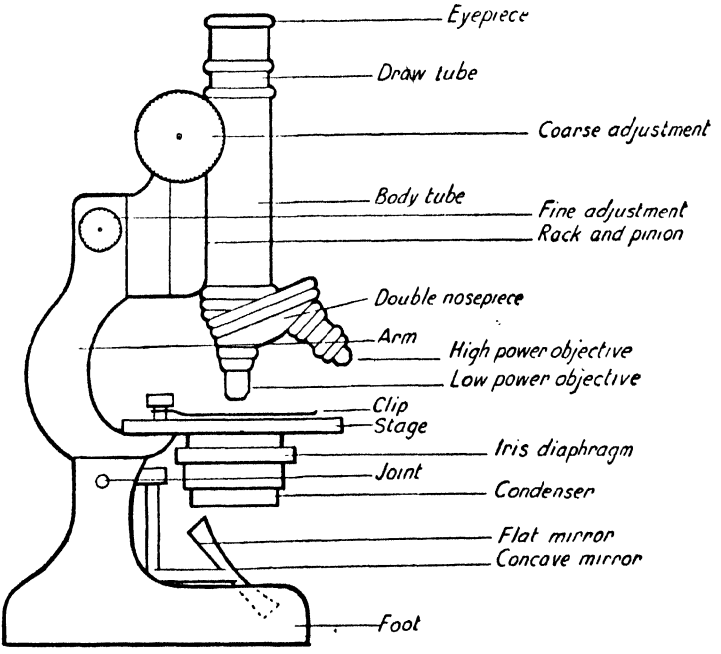

FIG. 2.—Parts of the Compound Microscope.

tube which carries the eyepiece. Under the stage is a **substage.**

The Lenses

The **eyepieces** or **oculars** most commonly used are numbered 1, 2, 3 or 4 and magnify respectively 5, 6, 8 and 10 diameters.

An eyepiece (Fig. 3) is a tube carrying an upper or **" eye " lens and a lower or " field " lens and containing**

a diaphragm at the focal point of the eye lens. The eyepiece may be fitted with a pointer or with cross wires for locating objects of special interest.

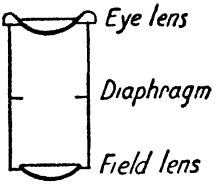

FIG. 3.—Sectional View of an Eyepiece.

The **objectives**, which are tubes each fitted with a system of lenses, are screwed into a rotating and dust-proof nosepiece so that either may be brought into position for use.

The **low power objective** (Fig. 4) has the larger external lens, and the one most commonly used is marked thus: $\frac{2}{3}$ in. (English), 16 mm. (American and some Continental makes). These figures indicate the focal length of the system of lenses and not the working distance, i.e. not the distance between the outer lens and the object when in focus, which is only about 7 mm. and thus less than the focal length.

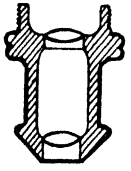
FIG. 4.—Sectional View of a Low Power Objective.

The low power objective usually magnifies the object about 10 times, but the primary image formed by it within the eyepiece is again magnified by the eyepiece lenses forming an enlarged secondary virtual image (see Fig. 1). Thus using a No. 2 eyepiece, × 6 and a two-third objective, we get a total magnification of 10 × 6 = 60 diameters.

Modern objectives are corrected for a definite length of body tube, usually 6 or 7 ins. (160 mm. or 180 mm.). To obtain this it may be necessary to pull out the draw tube a little, hence the latter should be graduated in inches or millimetres. Further extension of the draw tube increases the magnification of the primary image formed by the objective but does not increase the direct magnification of

the object, and so does not give any further details of structure.

The **high power objective** (Fig. 5) has the smaller external lens and is marked $\frac{1}{6}$ in. or 4 mm., the working distance being about 0.6 mm.

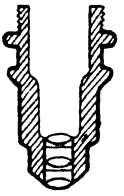

FIG. 5.—Sectional View of an High Power Objective.

The primary magnification is about 40 diameters, so that with a No. 2 eyepiece, × 6 and 160 mm. tube, the total magnification is 40 × 6 = 240 diameters.

The Illuminating Parts

The **two mirrors,** one plane and the other concave, are mounted back to back in a frame on a universal joint which allows them to be so placed that either can catch light from any direction and reflect it through the aperture in the stage. The mirror must be so arranged that its centre is on the optical axis of the microscope in order to act efficiently.

If the mirror is swung out so that its centre is not on the optical axis, oblique illumination results and shadows are cast upon the object. This must be avoided.

The flat mirror reflects parallel rays of light and is used with strong light or with a condenser. This is a system of lenses which concentrates the light coming from the mirror. It is not necessary to use it with low powers, but it can be used to advantage with $\frac{1}{6}$ in. objectives. The concave mirror also concentrates the light and so is used when a bright illumination is required, and the condenser is not in use.

The **substage and fittings.** Below the stage is a substage which contains an **iris diaphragm,** the aperture of which may be altered by rotating a collar. By modifying the

size of the aperture the amount of light passing to the objective may be modified and the clearness or definition of the image improved.

The substage also carries the **condenser** when one is fitted and is then provided with some form of adjustment for focussing the condenser on to the object.

The Focussing Adjustments

The **coarse adjustment** is actuated by a pair of large milled heads which rotate a spiral pinion whose teeth engage on a rack attached to the body tube. Turning the milled head so that its upper edge moves towards the observer raises the body tube.

The **fine adjustment** is actuated either by a single milled head with a vertical axis, or by one or two lateral milled heads with a horizontal axis.

Objects to be examined under objectives up to and including $\frac{1}{6}$ in. should always be found and focussed with the coarse adjustment. The fine adjustment is intended only for critically focussing parts of an object already in view, and the milled head should be turned backwards and forwards only, not right round.

§ 3. Preparation of Objects for Examination

Objects are usually examined by transmitted light, and in order that light may pass through them they must be very thin. Consequently, except in the case of powders, fibres or very small objects, thin slices or sections have to be prepared and rendered translucent. The smallest quantity of material which will show what it is desired to see should always be used.

An object to be examined is mounted on a glass slide

3 ins. by 1 in. and is covered with a thin sheet of glass called a coverslip.

Coverslips $\frac{7}{8}$ in. square, No. 2 thickness, are suitable for elementary plant anatomy.

A liquid called the mounting medium is nearly always

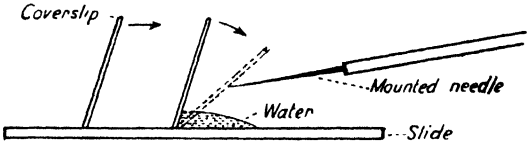

FIG. 6.—Method of Putting on a Coverslip.

included in the space surrounding the object between the slide and the coverslip.

EXERCISE 1.—TO MAKE A SIMPLE MOUNT OF A POWDER SUCH AS POTATO STARCH

1. Clean both slide and coverslip thoroughly. Dust or dirt on either may be mistaken for parts of the object and in any case prevent distinct vision.

2. Place a **very small quantity** of the starch—about enough to cover a pin's head—in the centre of the slide.

3. Catch a small drop of water from the tap on the end of a glass rod and mix the starch in this so that all the grains are thoroughly wetted and distributed.

4. Hold the coverslip vertically with its edges in contact with finger and thumb of left hand ; bring the lower edge down on to the left-hand end of the slide (Fig. 6) ; move the coverslip to the right until it touches the water drop : then, supporting its upper edge with a needle, allow it to fold down gently on to the water drop. In this way the inclusion of air bubbles, which prevent distinct vision, is avoided.

5. The coverslip should now adhere sufficiently firmly to the slide, so that if the latter is held at a slope the former will not

move. If, when this test is applied, the coverslip shifts, too much mounting fluid has been used, and a little should be drawn away by applying the edge of a piece of blotting-paper to the edge of the coverslip. If the mounting fluid does not fill the underside of the coverslip, touch the slide and edge of coverslip simultaneously with a drop of water on a glass rod; capillarity will cause water to run from the rod to fill the space.

6. Take great care that liquids do not get on top of the coverslip or under the slide. If they do so they may be transferred either to the objective, which is then rendered useless, or to the stage, in which case moving the preparation becomes difficult and damage is done to the microscope.

§ 4. Method of Work with the Microscope

Exercise 1*a*.—Setting up the Instrument

Position.—Place the microscope on the bench opposite the left * shoulder with the rear edge of the stage parallel with the front edge of the bench. Tilt the arm on the foot a little so that by nodding the head the left eye * comes naturally into position above the eyepiece.

Illumination.

(A) Without a Condenser

See that the low power objective (larger external lens surface) is in position; that the swinging rod of the mirrors is central and that the iris diaphragm is open. Hold the mirrors between the fingers and thumbs of both hands and move them on the universal joint (not the swinging joint) until the field of view appears brilliantly illuminated.

(B) With a Condenser

Proceed as in (A), being sure that the flat mirror is reflecting the light in this instance. Then, after obtaining a fair light,

* A left-handed person should read right for left.

place on the stage the object to be examined and focus it with the low power. Move the condenser up and down until the image of the light source appears accurately focussed on the preparation. Then slightly lower the condenser till the image of the source of light just fades out of view.

Once the microscope is set up it should not be moved bodily nor should the light source be shifted till work is concluded.

Exercise 1*b*.—Examination of the Object

Place the preparation to be examined over the aperture in the stage with the coverslip uppermost. Secure it by means of the clips.

Always examine with the low power first.

Focussing the Low Power.—Look at the side of the microscope and rack the objective down with the coarse adjustment until the external lens surface and its reflection seen in the coverslip nearly meet. Apply the right eye * to the eyepiece and turn the coarse adjustment upwards and towards yourself until the object comes into view. Now use the left finger and humb and turn the fine adjustment backwards and forwards to study the details and depths of the object.

It is essential to acquire the habit of keeping both eyes open.

Selection of Part for Higher Magnification.—Unclip the slide and shift † it until a suitable part of the preparation appears in the centre of the field of view. Clip the slide in this position. For a powder the part chosen should show typical particles fairly well separated. In a section it should be a representative area which is thin.

Changing Objectives.—Rack the tube up till both objectives are well above the level of the stage. Turn the double nosepiece till the high power " clicks " into position.‡

* Left for left-handed person.

† Note that the actual movement of the object is apparently reversed when viewed through the microscope.

‡ See * at foot of next page.

Focussing the High Power.—Using the coarse adjustment and looking at the objective sideways, bring it down until its external lens surface almost touches the coverslip.*

Now rack upwards with the eye at the eyepiece : very little movement of the coarse adjustment is necessary. If, after half a complete turn of the coarse adjustment the object is not in view, it has been missed : the objective has been carried up too far or the object is not directly underneath. Commence the whole focussing process again. Never attempt to focus downwards with the eye at the eyepiece. The object would be missed again and the objective ultimately forced on to the coverslip and both broken. Having found the object under the high power, study details and depths with the fine adjustment.

The starch grains resemble oyster shells. They are flattened and mostly oval or triangular with rounded angles, the hilum being nearer to the narrow end and the laminations eccentric. Compound grains with two or three grainlets and semi-compound grains are fairly common.

Exercise 1c.—Drawing

Place the paper by the side of the microscope opposite the right shoulder. Use the left eye † for the microscope and the right eye to view the paper, so that either object or drawing may be seen by transferring attention from one to the other, but without moving the head. Use the left hand on the fine adjustment. Thus, in order to avoid strain, which leads

* Microscopes are made so that after an object has been focussed under the low power, it is only necessary to turn the nosepiece and change the objectives for the object to appear in approximate focus under the high power. In laboratories where numerous microscopes are in use, however, interchange of objectives, repairs and replacements tend to derange their original adjustment. The above precaution is necessary when the worker does not know for certain that the adjustment of the particular instrument he is using is correct.

† For a left-handed person the positions are reversed, the microscop having been set up opposite the right shoulder.

to poor work, one eye is used for preliminary examination and the other for viewing the object during drawing.

Type of Drawing Required

It should be the aim of the student to portray on paper the features of the object in as clear a manner as possible. For this purpose **line drawings to a large scale** should be used. Attempts at shading usually result in indefiniteness and for this reason should be avoided. Do not use coloured pencils.

All parts of objects shown in drawings should be "labelled." Their names should be written at the side of the drawing, each name being joined to its appropriate part by a clear straight line.

Exercise 1*d*.—Microchemical Test for Starch

Refocus the low power on the starch. Place a drop of iodine solution on the left-hand side of the slide so that it just touches the edge of the coverslip. Then, while watching the starch, touch the right-hand side of the coverslip with a piece of blotting-paper. Water will be drawn out, iodine will run under the coverslip and will turn the starch blue.

The blue substance is iodide of starch and the process just performed is called "irrigating" with iodine.

Now irrigate the iodide of starch with caustic soda: it will be seen to swell, gelatinise and lose its colour.

Exercise 2.—Air Bubbles

Put a drop of water on the slide and allow a coverslip to drop horizontally on to it. Note the bubbles of air that are included. Focus one of the small bubbles and see that its surface appears as a black ring caused by the refraction of the light at the air-water surface.

§ 5. Common Faults, their Causes and Remedies in Sequence as they may appear

1. INSUFFICIENT LIGHT. May be due to:

High power in position by mistake	Change objectives.
Swinging rod of mirrors not central	Centre it.
Flat mirror being used with too weak a light or hollow mirror with condenser .	Change mirror.
Too far from or too near light source	Adjust distance.
Diaphragm shut . .	Open it.

SPECKS IN FIELD OF VIEW:

Dirt on objective or eyepiece	Rotate eyepiece in body tube.
If specks rotate . . .	Clean eyepiece.
If they do not . . .	Clean objective.*

3. THE OBJECT CANNOT BE FOCUSSED CLEARLY:

Coverslip not perfectly polished	Remove coverslip, repolish and replace.
Exterior of objective dirty *	Polish with a dry duster.
If no improvement, dirt may be soluble in water . .	Polish with a damp duster and dry off.
If still no improvement, dirt may be canada balsam	Polish with a duster moistened with xylol and dry off.

* Neither eyepieces nor objectives may on any account be taken to pieces; only the external surface of the lens is to be polished.

4. OBJECTS APPEAR TO BE LATERALLY ILLUMINATED AND OFTEN APPEAR SILVERY:

Swinging rod of mirrors not central	Centre it.

5. INSUFFICIENT LIGHT (see No. 1):

Preparation too thick . .	Prepare a new and thinner section.

§ 6. Essential Rules for Microscopy

1. The microscope must be straight on bench opposite left shoulder. (For left-handed people, right shoulder.)

2. Always see that the low power is in position first.

3. Do not move microscope or the light source after you have arranged the lighting.

4. Always use the low power first.

5. Always focus upwards with coarse adjustment for both low and high power objectives.

6. Never twist the fine adjustment right round, but only turn it backwards and forwards.

7. Keep both eyes open and use right eye for microscope during preliminary examination and left while drawing. (Vice versa for left-handed people.)

8. Select under the low power a portion of the object suitable for higher magnification.

9. Make and keep drawings of all objects examined. In the case of large objects, such as sections of stems, always first make a large outline drawing of the object, naming the parts, and then make a detailed drawing of representative parts, using the high power.

10. Keep coverslip, objectives and stage dry and clean.

11. Neither eyepieces nor objectives may be taken to pieces except by a qualified optician; only the external surfaces of the outer lenses may be cleaned.

12. Always leave the microscope in a clean and dry condition after use. Failing to do this spoils the work of the next student as well as damaging the microscope and objectives.

§ 7. Additional Exercises in Making Simple Mounts

The following material, except where otherwise stated, may be mounted and drawn on a large scale as that in Exercise 1, p. 18 *et seq.*

Exercise 3.—Wheat Starch

The grains are flat circular discs, with a central hilum and concentric laminations, which are very difficult to see until the grains are slightly swollen with caustic soda. There are very numerous minute starch grains in wheaten flour.

Exercise 4.—Pea or Bean Starch

The grains are kidney shaped or elliptical, with a long central hilum and concentric lamination. Very often a crack appears in the region of the hilum from which clefts radiate. The crack is due to the unequal distribution of water in the central and peripheral parts of the grains so that they contract unequally on drying and swell unequally on soaking.

Exercise 5.—Oatmeal Starch

The grains are large, compound and more or less spherical; they show a network marking due to the outline of the grainlets into which they very readily break up.

Exercise 6.—Floor Sweepings

Many foreign objects frequently seen in microscopical preparations may be found in sweepings.

Fibres of:—

Cotton, which appear as flattened twisted tubes with rounded ends.

Linen, which have pointed ends, never twist and are seldom flattened.

Silk, which appear as solid rods with a more or less smooth surface.

Wool, which appear as solid rods, but have an outer coating of overlapping scales.

Pine wood, which have characteristic button-shaped marks on their sides.

Mineral particles of various shapes and colours, all recognisable by their angular faces.

Tiny particles of dust which are too small for their shape to be seen but which, on account of the fact that they are suspended in a fluid, are "wobbling" in Brownian movement.

Exercise 7.—Scale of Onion Bulb

Tear an onion scale obliquely and from the skin of the inside of the scale, which will project from one of the torn edges, cut out with a pair of scissors a piece about 1 cm. square. Mount this in water.

Examine under the low power and draw a few of the close-fitting cells, showing the cell wall and the nucleus of each.

Irrigate with iodine; focus under the high power and draw one cell and label to show:

The wall and its attachments to surrounding cells.

The cytoplasm.

The nucleus suspended by cytoplasmic strands.

The vacuole.

Exercise 8.—Leaf of *Elodea Canadensis*

Mount a single leaf of *Elodea*, upper side down, in a drop of the warm water (about 27° C.) in which the plant has been kept for half an hour. Find one of the cells at the edge of the leaf that projects as a tooth. Examine with the high power

and find the nucleus. Draw on a large scale the projecting cell and label the drawing to show the cell wall, cytoplasm and nucleus.

Notice the chloroplastids in most cells of the leaf. From one aspect they appear as green discs ; edge on view shows them to be biconvex. Stages in the fission (division) of the chloroplastids may perhaps be seen.

Find the elongated cells in the midrib of the leaf. Focus into them with the high power. Notice the rotation of the chloroplastids, which are carried round in the moving cytoplasm.

Observe that the direction of movement differs even in adjacent cells and in each cell there is a central neutral zone without movement. Draw two adjacent cells and indicate direction of movement by arrows.

Exercise 9.—Filaments of Living *Spirogyra*

Mount a few threads of *Spirogyra* in water.

Examine under the low and the high power of the microscope and draw one cell as seen under the high power.

Irrigate with iodine and look for grains of iodide of starch round the pyrenoids.

Exercise 10.—Cells of Rose Fruit

Remove a piece of skin from a ripe rose fruit. Scrape some of the cells from the inner layers of the skin into water on a slide and cover with a coverslip. Observe and draw a few selected cells with chromoplastids.

CHAPTER II

CUTTING AND MOUNTING SIMPLE SECTIONS— MICROCHEMICAL REACTIONS

IN order to study the internal construction of plant organs very thin slices or sections are cut with a razor and viewed under the microscope by transmitted light.

§ 8. The Razor

The kind of razor most suitable for section cutting is one sold specially for the purpose.* It is of the old "cut-throat" type, but differs in three essentials from the one used by the barber. It is a much weightier instrument, a quality which assists its passage through hard materials; one side of the blade only is hollow ground, the other being flat so that the blade travels straight; it is made of fairly soft steel so that when the edge is spoiled in use a new edge may be readily ground on it.

Sharpening the Razor

The razor should be sharpened every time it is used, so that the edge never gets even slightly dull. For this purpose a fairly fine hone or oilstone and a strop are necessary.

EXERCISE II.—CARRY OUT THE PROCESS:

1. Wet the stone with dilute glycerine or oil† and set it lengthways along the right-hand ‡ edge of the bench.

* Most botanical razors are ground for use in the right hand; left-handed razors can be obtained.

† One or the other must always be used on one particular stone.

‡ For left-handed razors read left for right throughout instructions.

2. Open the razor so that the handle continues the line of the blade.

3. Lay the flat side of the blade flat on the stone at its far end so that the length of the razor makes an angle of about 45°

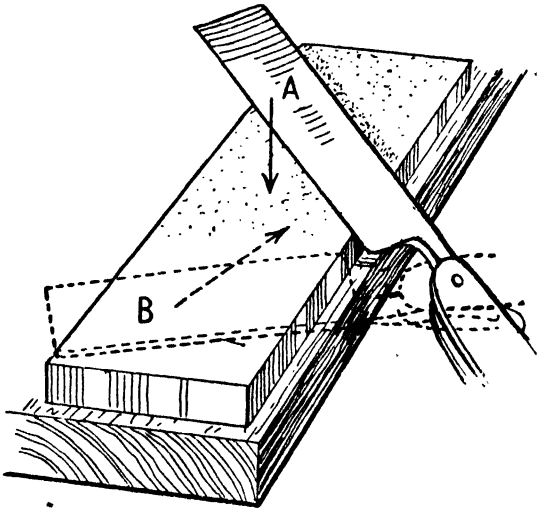

FIG. 7.—Method of Honing the Razor.

with the stone and the heel of the edge points towards you. Fig. 7, *A*.)

4. Pull the blade along to the near end of the stone, at the same time pressing the blade gently but firmly * with the fingers of the left hand to keep it flat on the stone.

5. Using the back of the blade as a fulcrum, roll the razor over so that the hollow side comes to lie flat on the stone; pivot the razor at the heel so that it again lies at 45° to the stone and the heel points away from you (Fig. 7, *B*).

6. Push the blade to the far end of the stone, keeping it flat

* Heavy pressure will lead to a wire edge which is detrimental to section cutting.

as before by gentle, and this time slightly lighter, pressure from the fingers of the left hand.

7. Roll the razor on its back and readjust into position as in 3 above, and then repeat 3 to 7 as often as is necessary.*

8. Finish off the edge by a similar application on the strop, except that in this instance the back of the razor travels first.

On the Stone.	*On the Strop.*
Razor flat.	Keep strop stretched tight.
Heel of edge leading.	Razor flat.
Do not press hard.	Back leading.
Roll razor on its back.	Press moderately hard.
	Roll razor on its back.

Holding the Razor for Cutting

Bend the handle of the razor on its pivot so that it takes on an angle of 90° with the blade and so that the edge of the blade is directed towards you and the main mass of the handle away. Grasp the razor with the tips of the index and middle fingers and thumb on the metal to the left of the handle and the third finger on the slight hook which continues the blade to the right of the handle.

§ 9. Cutting, Selecting and Mounting a Section

For first attempts use a piece of fresh potato tuber about 1 × 1 × 5 cms. in dimensions.

Keep the material thoroughly wet with water. Any drying of the material will lead to air bubbles in the section, which are to be avoided.

Holding the Material for Cutting

The material should be held between the index finger and thumb of the left hand, with the finger as straight as

* The edge should be sharp enough to shave hairs from the back of the hand or wrist when it is fit for using in section cutting.

possible, the material perfectly vertical and projecting a few mm. above the level of the finger, and the thumb as far as possible below (Fig. 8).

Exercise 12.—Cutting Fresh Potato Tuber

Holding the razor and the material as explained above, flood both with water. Then lay the flat of the blade flat on the

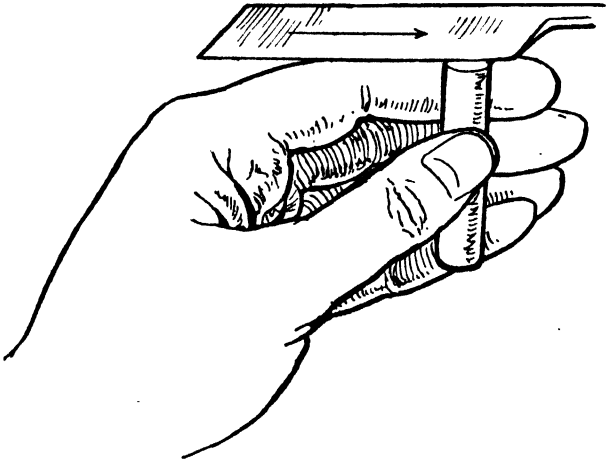

Fig. 8.—Method of Holding Material for Cutting Transverse Sections.

index finger of the left hand (Fig. 8) with the heel of the edge touching the side of the material. Draw the razor from left to right with a long slicing action, using the elbow and shoulder joints but keeping the wrist stiff. Do not use the razor as a chisel.

A slab of material should be removed to expose a flat face. This operation is called "trimming off the material."

Do not alter the grasp of the material.

Now lay the razor flat on the index finger again, but this time have the edge of the blade covering about half of the trimmed surface of the material (Fig. 8).

Repeat the slicing action, pressing the flat of the razor on to the index finger to steady it and thus remove a very thin slice or section of the material.

Obtain in this way a number of sections not more than a few sq. mm. in area.

EXERCISE 12*a*.—SELECTION OF SECTION FOR THINNESS

When a fair number of sections have accumulated on the razor, slide them off into a shallow dish of water.

Stir the liquid and while it is still in motion look for a shadow on the bottom of the dish that is more obvious than the section which casts it. Then find this section.

EXERCISE 12*b*.—MOUNTING THE SECTION

Carefully transfer the selected section by means of a section lifter or camel-hair brush to the middle of a perfectly clean slide. Put on a drop of dilute glycerine and a coverslip as in Exercise 1, p. 18.

EXERCISE 12*c*.—DRAWING

Make a clean-cut line drawing of the section as seen under the low power to show the shape and relationships of the cells and the distribution and shape of the starch grains.

Also draw one grain as seen under the high power.

§ 10. Additional Exercises in Cutting Simple Sections

Cut and select sections of the following material in the way described in Exercise 12, p. 31 *et seq.*, but if the material has been preserved in spirit use 25% spirit instead of water for flooding the razor and the material while the sections are being cut, and for immersion of the sections during selection.

EXERCISE 13.—ORCHID (*Phajus grandiflora*) OR POTATO TUBER, OR IRIS RHIZOME SUITABLY PRESERVED

Mount selected sections in water.

Observe the short rod-shaped leucoplastids often clustered

round the nucleus in the outer cortical cells. In the inner cells look for stages in the formation of large starch grains, each fully formed grain having a large rod-shaped leucoplastid attached to its broad end.

Irrigate with iodine.

Protoplasmic structures including leucoplastids go yellow to brown ; starch goes blue.

Draw cells containing leucoplastids only and others showing stages in the formation of starch grains.

EXERCISE 14.—*Pellionia pulchra* STEM

Cut sections as nearly as possible at right-angles to the length of the stem. Mount in water.

Examine closely under the high power and observe the small chloroplastids in the outer cortical cells and those in the inner cells, each developing a starch grain. In the cells nearest the centre the fully formed starch grains appear to have a green cap (actually the chloroplastid) at the broad end.

Irrigate with iodine.

Draw stages in the formation of starch grains.

§ 11. Microchemical Reactions

Many structures seen under the microscope may be recognised by their shape, but in order to be perfectly certain as to the material of which they are composed, chemical tests are performed upon them which give definite changes with particular substances.

Thus, if a colourless oval granule thought to be starch is treated with iodine and a blue to black colour results, the assumption becomes definite knowledge. This principal microchemical test may be followed by a confirmatory test. When the blue iodide of starch is treated with caustic soda or caustic potash, swelling and loss of colour confirm the presence of starch. Sometimes two reagents

are used one after the other to produce the primary result. Sometimes one section is used for the primary test and a second section has to be treated differently for confirmation.

Table of Principal Simple Microchemical Tests

In the table given below, the method of application and the reagent are followed by the change in colour or other change which indicates the presence of a particular substance.

Numbers following the indicated substance refer to the number of the confirmatory test which should be applied. Numbers following the method of application indicate that the same section from the test so numbered should be used again. Otherwise a new section should be used for each test.

In all cases the reagent should be applied to the section on the slide and it should be covered with a coverslip before it is examined under the microscope.

Method of application.	Reagent.	Positive result.	Indicating.
1. Mount in ..... Examine (quickly in the case of preserved material) for ...	water.	Crystalline cell contents	Inulin, Calcium carbonate, Calcium oxalate (2)
Section will also show	.....	.....	Cell walls (10)
which may enclose	.....	.....	Liquid contents only (5, 6, 19)
or.....	.....	.....	Homogeneous solid contents (8)
or	.....	.....	Contents not homogeneous (10)
2. Heat (1)	.....	Crystals dissolve ...	Inulin (21)
		Crystals do not dissolve	..... (3)
3. Remount (2) in	dilute acetic acid ..	Crystals dissolve ...	Calcium carbonate.
		Crystals do not dissolve.....	..... (4)

Method of application.	Reagent.	Positive result.	Indicating.
4. Soak in.....	water, 3 mins.		
Drain off and mount in.....	75% sulphuric acid	Crystals dissolve and reprecipitate as small needles	Calcium oxalate.
5. Soak sections of fresh material in	absolute alcohol ...	Precipitate in cells .	Sugar or Inulin (19)
6. Mount in	ferric chloride solution	Blue-black or green colour	Tannin (7)
7. Mount in	mixture of iodine and 10% ammonia ...	Bright red colour ...	Tannin confirmed.
8. Mount in	tincture of alkanet .	Red or pink colouration	Resin (9) or Oil (15)
9. Soak in.....	90% alcohol	Material dissolves ..	Resin confirmed.
10. Mount in	iodine	Blue to black ..... or Yellow to brown ...	Starch (11) Protein (12 and 13) Lignified wall (14) Cuticularised wall (15) Suberised wall (15)
11. Remount (10) in	caustic soda solution	Loss of colour and gelatinisation ...	Starch confirmed.
12. Cover with	strong nitric acid and warm *		
Add	strong ammonia ... (Xanthoproteic test)	Orange yellow	Protein confirmed (13)
13. Mount in	Millon's reagent and heat	Brick red	Protein confirmed.
14. Soak in.....	phloroglucin, 3 mins.		
Drain off excess and add	strong hydrochloric acid.		
Drain off excess and mount in.	glycerine	Red to purple	Lignified walls confirmed (possibly also suberised walls) (15)
15. Mount in	sudan III	Slowly red	Suberised or cuticularised wall confirmed (17) Fat or Oil in the cell (16)
16. Irrigate (15) with ether. Examine quickly	.....	Red drops dissolve..	Fat or Oil confirmed.
17. Soak in	iodine, 3 mins.		
Drain off and mount in	75% sulphuric acid	Blue and swelling .. Brown, but no swelling even after some hours	Cellulose wall (18) Cuticularised wall confirmed.
18. Mount in	chlor-zinc iodine ... (Schultze's reagent)	Blue-violet	Cellulose wall confirmed.
19. Cover with .... and heat	Fehling's solution	Red precipitate	Glucose, Fructose or Maltose.
		No red precipitate..	..... (20)

* Care must be taken that the fumes of this acid do not come into contact with the microscope.

Method of application.	Reagent.	Positive result.	Indicating.
20. Cover with.....	dilute hydrochloric acid.		
Heat.			
Neutralise with..	strong caustic soda.		
Cover with	Fehling's solution		
and heat	.....	Red precipitate	Sucrose or Inulin (21)
21. Cover with.....	10% solution of *a*-naphthol.		
Add	few drops of strong sulphuric acid.		
Warm.....	.....	Deep violet.	Inulin confirmed.

§ 12. Exercises in Simple Micro-analysis

Cut and select sections of the following material in the way described in Exercise 12, pp. 31 and 32, but if the material has been preserved in spirit, use 25% spirit instead of water for flooding the razor and keeping the material wet, or, if the material has been preserved in the dry condition, cut it with a dry razor.

Having obtained positive results from the tests enumerated in the exercises, be sure to apply the confirmatory tests indicated in the table, pp. 34–36.

Record the methods used, make notes on the results obtained and deductions made in a table similar to that given in § 11, pp. 33–36.

Exercise 15.—Fresh Apple, Pear or Grape

Treat fairly thick sections as directed in the table, pp. 34–36, Tests 1, 5, 19 and 17.

After 19, if the section is gently washed and mounted in water or glycerine the granules of copper oxide will be seen in the cells which contained sugar.

Exercise 16.—Fresh Parsnip

Treat sections as directed in the table, pp. 34–36, Tests 1, 5 and 19. Note the results.

EXERCISE 17.—ARTICHOKE (*Helianthus tuberosus*) TUBER, FRESH

Treat sections as directed in table, pp. 34–36, Tests 1, 5, 19, 20, 21. Note the results.

EXERCISE 18.—ARTICHOKE (*Helianthus tuberosus*) TUBER, PRESERVED

Treat sections as directed in table, pp. 34–36, Tests 1, 2, 21. Note the results.

Also mount a section in glycerine and make a drawing under the low power to show distribution of the sphærocrystals of inulin in the cells.

EXERCISE 19.—PEA OR BEAN SEED, PRESERVED IN SPIRIT

Remove the seed coat and cut sections at right-angles to the flat face of the cotyledon. Treat as directed in table, pp. 34–36, Tests 1, 10, 11, 12, 13. Note the results.

Also mount a thin section in dilute glycerine and observe the mass of small rounded protein granules in which the starch grains are embedded.

EXERCISE 20.—ALMOND, DRY

Mount a section in water, warm and observe the drops of oil in and around the section. Treat other sections according to the table, pp. 34–36, Tests 10, 12, 13, 15 and 16. Note the results.

EXERCISE 21.—CASTOR OIL (*Ricinus communis*) SEED, DRY

Break the brittle seed coat from one end only of the seed. Cut sections of the endosperm with a dry razor and treat as in Exercise 20. Note the results.

Also dissolve out the oil from a number of sections with absolute alcohol in a watch glass.

Mount one section in dilute glycerine and observe the sharply defined crystalloid and globoid in each aleurone grain,

the outer layer of the grain having swollen up and become transparent.

Mount another section in glacial acetic acid and observe the crystalloids swell up and disappear while the globoids appear alone for a time and then dissolve.

Exercise 22.—Brazil Nut or Walnut, Dry

Remove the outer shell and cut sections of the food reservoir. Treat as in Exercise 21, p. 37. Note results.

The aleurone grains have crystalloids but no globoids.

Exercise 23.—Sunflower (*Helianthus annuus*) Fruit, Dry

Treat as in Exercise 21, p. 37. Note results.

The aleurone grains have no crystalloids.

Exercise 24.—Annual Lupin (*Lupinus hirsutus*) Seed, Dry

Cut a seed across with a sharp penknife, and so expose a flat face. Make the usual section-cutting actions, with a dry razor, applying firm pressure but not force to the cut surface of the dry seed, and a roll of tissue will be removed.

Transfer this to a drop of water on a slide. The roll will uncurl and become flat.

Proceed in the same way to prepare other sections for examination according to table, pp. 34–36, Tests 10, 12, 13, 17 and 18. Note results.

Examine, also, a section mounted in dilute glycerine and observe the thick cell walls; the intercellular spaces (black, owing to the optical effect of the included air); and the simple tablet aleurone grains.

Exercise 25.—*Tetragonia crystallina* or *Myriophyllum* Stem

Cut sections of the stem a few sq. mm. in area as nearly as possible at right-angles to the length.

Mount in water, draw cells to show the cluster crystals and

treat sections according to the table, pp. 34–36, Tests 1, 2, 3 and 4. Note results.

EXERCISE 26.—HYACINTH (*Hyacinthus orientalis*) BULB, SCAPE OR LEAVES

Cut a bulb or a piece of scape vertically down the middle and then cut sections parallel with the surface so exposed, or hold four or five pieces of hyacinth leaf in a bundle and cut sections parallel with the length of the leaf. Treat as in Exercise 25, p. 38. Note results. Draw cells containing raphides.

EXERCISE 27.—BELLADONNA (*Atropa Belladonna*) ROOT

Cut sections at right-angles to length. Treat as in Exercise 25, p. 38. Note results. Find cells showing innumerable minute crystals (crystal sand).

EXERCISE 28.—*Kleinia hastata* OR LIME (*Tilia*) STEM

Cut sections of the outer tissues parallel with the length of the stem. Draw cells containing single tablet crystals and treat as in Exercise 25, p. 38. Note the results.

EXERCISE 29.—*Sedum spectabile* STEM

Cut sections a few sq. mm. in area at right-angles to the length. Treat as in the table, pp. 34—36, Tests 1, 10, 14, 15, 17 and 18. Note results.

EXERCISE

EXERCISE

EXERCISE

This space is left blank for notes on exercises other than those given above.

§ 13. Conventional Methods of Cell Wall Drawing

In order to avoid obscurities and complications in drawings the student is advised to follow certain conventions in drawing cell walls.

(*a*) A cellulose wall unless it is very thick should be represented as one line, the thickness of the wall being ignored.

(*b*) A very thick cellulose wall such as occurs in collenchyma should be represented by a breadth of paper between two suitably spaced lines. In effect the cavities of the contiguous cells are delineated.

(*c*) A lignified wall should be represented in a similar manner, but distinguished from a thick cellulose wall by a median line in the position of the middle lamella of the wall which is usually visible in the section.

EXERCISE 30.—ELDER (*Sambucus nigra*) STEM, YOUNG

Cut a very thin section as nearly as possible at right-angles to the length of the stem. Treat with phloroglucin and hydrochloric acid (Microchemical Table, p. 35, test 14), find under the low power, focus under the high power, and draw groups of:

Three or four non-coloured cells with thin cellulose walls from the middle of the stem.

A few of the cells with thick cellulose walls that are to be found in patches just under the surface.

A few of the "red," lignified elements.

CHAPTER III

CUTTING, MOUNTING AND DRAWING STEM SECTIONS

§ 14. Types of Section

SECTIONS of stems may be cut with advantage in three planes (Fig. 9).

(*a*) Transverse sections, in the plane at right-angles to the length of the stem.

(*b*) Longitudinal radial sections, parallel with the length of the stem, with one edge of the section forming a radius of the transverse section.

(*c*) Longitudinal tangential sections, parallel with the length of the stem and at right-angles to (*b*), but not passing through the centre.

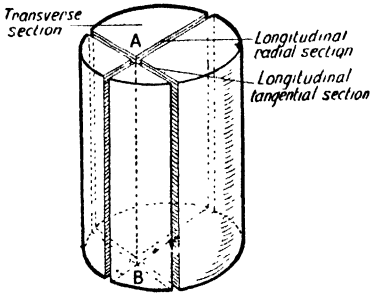

FIG. 9.—To illustrate the Planes of the Three Types of Section.

Transverse sections are of value in studying the general distribution of the tissue elements in relation to one another. Longitudinal sections must be made for the purpose of studying the individual element and the distribution and construction of such structures as medullary rays.

Nearly every element in a stem is so constituted that certain of its walls are in the transverse and certain in the longitudinal planes. Since the thinnest section has some

thickness, unless it is in one or other of these planes it will not appear distinct under the microscope.

In truly transverse sections the longitudinal walls of the elements stand vertical on the slide, and hence the part of the wall that is not in focus at the moment will be masked by the part that is. Thus provided a section be perfectly transverse it may be fairly thick and yet be clearly seen.

On the contrary, in a section which is even slightly oblique, the longitudinal walls stand sloping on the slide. As a consequence, no matter at what level the microscope is focussed, parts that are in focus will be blurred by those which are not.

Truth of direction of sections depends primarily on the accuracy with which the material is trimmed and subsequently on maintaining the trimmed surface true. This latter can best be accomplished, when transverse sections of cylindrical objects such as stems or roots are to be cut, by rolling the material between the finger and thumb a quarter of a complete turn after each section is removed.

Comparison of Transverse and Oblique Sections

EXERCISE 31.—SEDUM (*Sedum spectabile*) STEM

Trim off the end of a piece of Sedum stem at right-angles to the length (Exercise 12, p. 31) and cut a number of perfectly transverse sections. Float the sections off the razor into a dish of 25% spirit.

Deliberately trim off the end of the stem to form an angle of about 60° with the length and then cut sections parallel with this sloping surface. Float the sections off into a second dish of 25% spirit.

From the two batches select two sections which appear equally thin (Exercise 12*a*, p. 32). Transfer both to the middle of one slide and treat with phloroglucin and hydro-

chloric acid (§ 11, pp. 33–36, test 14), and mount in dilute glycerine under one coverslip.

Examine under the low power and note :

(*a*) That the oblique section appears blurred in comparison with the transverse one.

(*b*) That as the focus of the microscope is altered the oblique section appears to shift laterally while the truly transverse one does not.

Examine under the high power and note :

(*a*) That cell wall edges (and perhaps the surfaces of a few end walls) only are visible in the transverse section.

(*b*) That the sloping side walls can be seen in the oblique section.

This knowledge should be applied in selecting every transverse section for examination.

§ 15. Tissue Studies

Exercise 32.—Marrow (*Cucurbita Pepo*) Stem

Cut a number of truly transverse sections of Cucurbita stem. Select one for thinness (Exercise 12*a*, p. 32) and truth of direction (Exercise 31, p. 42). Treat with phloroglucin and hydrochloric acid and mount in dilute glycerine (§ 11, Test 14). Examine under the low power.

A ring of red elements just within the edge of the section are sclerenchyma fibres. Groups of red elements deeper in are xylem vessels. Find some rounded thin-walled cells between the sclerenchyma and the xylem. They are parenchyma (Fig. 10). Note that although the general appearance of the cells is circular, where contact between them occurs the walls are flat. The only part of the wall that is really curved is that which bounds an intercellular space.

Draw a few cells, using the single line to indicate the thin cellulose wall. Label the drawing.

Find some thick-walled colourless collenchyma (Fig. 11) just inside the surface. Focus under the high power. Draw

by outlining the cell cavities and allowing paper surface between to represent the thick cellulose walls. Small angular cavities are intercellular spaces. Label the drawing, which must be to a large scale.

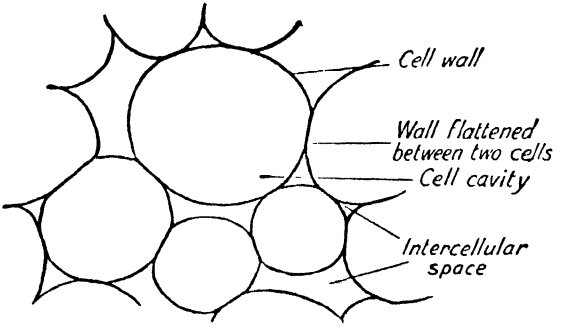

FIG. 10.—Parenchyma Cells from Ecballium Stem. × 275 approx.

Focus the sclerenchyma under the high power (Fig. 12). The walls are thick and have running through them a central layer of different optical properties to the main mass of the wall. This layer shows as a line in transverse sections and is called the middle lamella.

Draw the middle lamella first very lightly (Fig. 12, *a*). (In

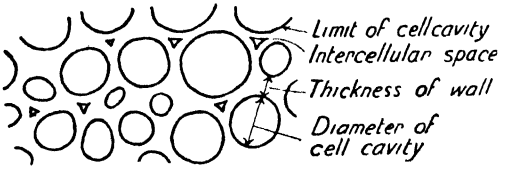

FIG. 11.—Collenchyma Cells from Ecballium Stem as seen in Transverse Sections. × 275 approx.

places where it is not obvious it may be assumed to run through the middle of the wall.) Then outline the cell cavity so that your finished drawing resembles Fig. 12*b*, p. 45, taking care to get the correct proportion between the diameter of the cell

cavity and wall thickness. Label the drawing, which must be large.

Find the phloem (Fig. 13) just outside the xylem. The

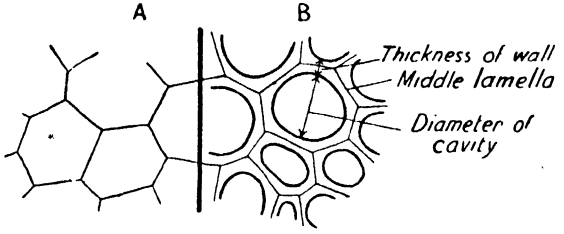

FIG. 12.—Two Stages in drawing Sclerenchyma Fibres as seen in a Transverse Section of Ecballium Stem. × 275 approx.

walls are of cellulose and relatively thin, hence they are to be represented as single lines. There are no intercellular spaces, so the elements are polygons whose corners meet. In this instance the sieve tubes are large, companion cells small and phloem parenchyma cells of intermediate size.

In drawing commence with one polygonal element. Then

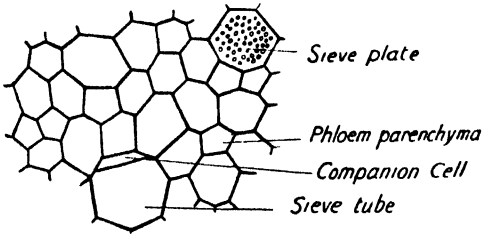

FIG. 13.—Phloem as seen in a Transverse Section of Ecballium Stem. × 275 approx.

draw the walls of the contiguous elements that project from its corners. Complete the elements so partly drawn and repeat the process. Label the drawing, which must be large.

The xylem consists of xylem vessels and other elements (Fig. 14). The latter may have lignified walls or cellulose ones. They may be tracheids or lignified parenchyma cells or non-lignified parenchyma. All the elements are more or less polygonal.

To draw:

Sketch in lightly the large xylem vessels (Fig. 14, *a*). Draw out from them, in the correct positions, lines for the

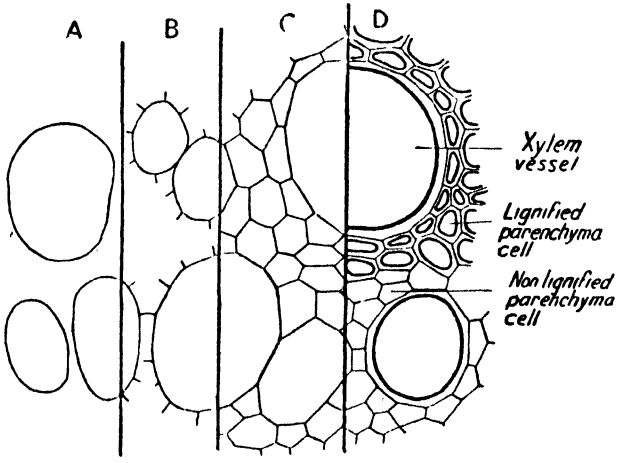

FIG. 14.—Stages in Drawing the Xylem of Ecballium Stem as seen in Transverse Section. × 275 approx.

radiating walls of the non-lignified contiguous elements or for the middle lamella of lignified ones (Fig. 14, *b*).

Flatten off the faces of the outside of the xylem vessels by drawing chords between the inner ends of the radiating walls of the contiguous elements.

Complete the latter and continue as for phloem (Fig. 14, *c*).

Then draw in the line representing the inner surface of the wall of all lignified elements, taking care that the true proportions are maintained so that your finished drawing resembles Fig. 14*d*. Label the drawing, which must be large.

EXERCISE 33.—MACERATED SUNFLOWER (*Helianthus annuus*) STEM

Mix a very small drop of macerated sunflower stem with dilute glycerine on a slide and put on a coverslip.

Find and draw on a large scale the following isolated elements:

A parenchyma cell. Some will appear almost round, but most are cubical.

A sclerenchyma fibre, which may be recognised by its long narrow shape, its sharp pointed ends and its very thick wall.

A length of pitted vessel. Vessels usually break up into lengths during the process of maceration. These are recognised by their tubular shape and the numerous spots—pits—in their walls.

A length of spiral vessel, which resembles a length of spiral spring.

A length of annular vessel, which may be recognised by the separate rings of thickened wall. Sometimes maceration causes solution of the cellulose tube in which these are set and the rings become isolated.

EXERCISE 34.—MARROW (*Cucurbita Pepo*) STEM

Cut longitudinal tangential sections* by shaving away a short length of one of the ridges of the stem. Treat some with phloroglucin and hydrochloric acid and mount in dilute glycerine. Mount others in magenta and cotton blue in lactophenol.†

The sections from nearest the surface will show collenchyma, parenchyma or sclerenchyma. Those from deeper in, that have passed through the vascular bundle (seen as a whitish streak), will show phloem or xylem.

Search for sclerenchyma and xylem in the phloroglucin preparation and make large labelled high power drawings of a few elements of each.

In the magenta and cotton blue preparations search for

* See Fig. 18, p. 60. † See § 16*a*, p. 49.

regions which show dense blue transverse blobs. These are sieve plates. Draw, under the high power, to a large scale, one or two sieve tubes with their companion cells, and label the drawing.

EXERCISE 35.—BOTTLE CORK

Cut a very thin section of bottle cork. Soak in alcohol to remove the air and then mount in sudan III. Note that the walls gradually pick up the colour of the stain, orange yellow to red (Microchemical test for suberised walls).

§ 16. Colouration of Sections

In order to make the various structures in a section more readily visible, and also to assist in distinguishing the kinds of elements, a section is often treated in such a way as to colour some or all of them.

TEMPORARY PREPARATIONS

The microchemical reactions listed in § 11 may be used for this purpose. They yield results which are diagnostic of the facts to which they apply, but the preparations so made cannot be kept in a satisfactory condition for more than a few days. Hence such preparations are called temporary preparations.

DOUBLE STAINED AND PERMANENT PREPARATIONS

Other methods of treatment entail the staining or dyeing of the substance in the section, and in many instances different material in the same section may be stained contrasting colours. The result is a "double stained preparation." The colourings obtained by these methods are seldom of accurate diagnostic value, but such preparations may usually, by suitable subsequent treatment, be made into "permanent preparations" which will keep for a period of years.

(*a*) Magenta and Cotton Blue Method

A very thin section is mounted in a solution of two dyes, magenta and cotton blue in lactophenol.*

Almost immediately, protein, protoplasm and mucilage stain blue, lignified walls always, and suberised walls, sometimes, stain red slowly and cellulose walls stain blue ultimately.

The section may be examined and drawn while still in the stain mixture, and this is advisable since staining improves as the section soaks.

The section may be kept, in a horizontal position, mounted as it is in the mixture which has no tendency to dry, and in about 7 to 14 days' time staining will be at its best. The preparation may then be made permanent.

(*b*) To Make a Magenta and Cotton Blue Preparation Permanent

1. Remove the coverslip, wipe away as much stain as possible without touching the section or letting it dry.

2. Put a few drops of methylated spirit on one end of the slide ; tip it backwards and forwards over the section and then off the slide into the sink.

3. Repeat this process a number of times, substituting absolute alcohol for methylated spirit in the last two washings. This should remove all traces of water. (Dehydrate.)

4. Give one final rinse with absolute alcohol and quickly cover the section with a drop of xylol.

The section should become much more transparent as the oily liquid soaks into it and hence the process is called clearing.

* *Ann. Bot.*, XLVIII, No. CLXXXIX, Jan. 1934.

5. Put a drop of canada balsam on to the section and then cover it with a coverslip.

6. Stick a label on one end of the slide and on it write a brief description of the object mounted.

7. Keep the slide horizontal till the balsam has set.

(*c*) The Safranin and Light Green Method

1. Soak a very thin section in a few drops of safranin on a slide for 5 minutes. If the stain, which is dissolved in 75% spirit, tends to dry, add a little more.

2. Tip off the excess stain.

3. Put a few drops of methylated spirit on one end of the slide ; run it backwards and forwards over the section and then tip it off into the sink.

4. Repeat 3, using absolute alcohol, till a quick glance at the section under the low power of the microscope shows that the red dye has been nearly removed from all except the lignified walls.

5. Give one final rinse with absolute alcohol and quickly cover the section with a drop of light green in clove oil and a coverslip. The light green dye will gradually stain the cellulose walls and the oil clears the section.

The section may now be drawn and will keep in this condition for a few days. It should not be kept too long in the light green in clove oil or the red will be masked by excessive green staining.

(*d*) To Make a Safranin and Light Green Preparation Permanent

1. Lift the coverslip.

2. Rinse off the oil of cloves with xylol.*

* Unless the oil of cloves is completely removed the preparation will fade.

3. Carefully wipe away excess xylol.

4. Quickly put a drop of canada balsam on top of the section.

5. Put on a coverslip and keep the preparation horizontal till the balsam has set.

6. Stick a label on one end of the slide and on it write a brief description of the object mounted.

FAULTS WHICH MAY APPEAR IN DOUBLE STAINED PREPARATIONS

Method.	Fault.	Cause.	Cure.
Magenta and cotton blue.	Sufficient light cannot be made to penetrate a temporary mount.	Section is too thick.	Cut a thinner section.
	Differential staining does not occur.	Section not left long enough in stain.	Remount the section in the stain mixture.
		The method is not suitable for the particular material.	Treat another section in another method.
Safranin and light green.	Safranin remains in non-lignified elements.	Incomplete washing with absolute alcohol.	Repeat the process.
	Safranin persists after repeated washing with absolute alcohol.	Cells contain tannin or mucilage.	Ignore.
	The removal of safranin from non-lignified elements is accompanied by its removal from lignified ones.	Section is too thick.	Cut a thinner one.
		Section not left long enough in safranin.	Commence staining again.
Magenta and cotton blue, or safranin and light green.	Whole section in xylol or oil of cloves appears whitish to the naked eye and cellulose walls blue-black or green-black under the microscope.	Water in section. (*a*) Incomplete dehydration, or (*b*) Breathing on section after dehydration.	Repeat washings with absolute alcohol and restain if necessary.
	Patches of the section have the appearance noted above.	Slide was not dry.	Repeat dehydration and use clean dry slide.
	Air bubbles in section.	Section allowed to dry or to float on top of one or other of the liquids used.	Soak section for some hours in absolute alcohol or prepare a new one.
Mounting in balsam.	Air bubbles in balsam.	Balsam stirred too vigorously.	Prick bubbles with a hot needle.
	White patches in balsam.	Slide not dry.	Use another.

(*e*)

(*f*)

This page is left blank for notes on other methods of staining which may be preferred to those given in the preceding pages.

§ 17. How to Study Transverse Stem Sections

For first attempts use pieces of Sunflower (*Helianthus annuus*) or Dahlia stem that have been selected to show the primary structure and have been preserved in 75% spirit.

Keep the material immersed in 25% spirit when it is not actually being cut, and while it is being cut keep both the material and the razor blade wet with the same liquid. In studying any structure always examine gross features first and work through to smaller and smaller detail.

Hand Lens Study

EXERCISE 36.—PREPARING A SLICE FOR HAND LENS STUDY

Trim off the end of a piece of stem at right-angles to its length.

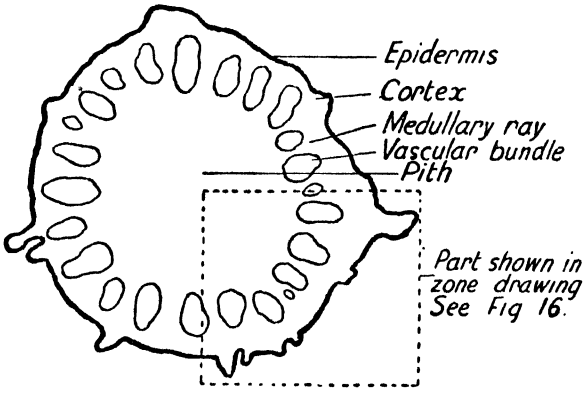

FIG. 15.—Hand Lens Drawing of Transverse Section of Sunflower Stem. × 10 approx.

Cut a fairly thin slice right across the stem ; transfer it to the middle of a slide, treat it with phloroglucin and hydro-

chloric acid (§ 11, pp. 33–36, test 14), and mount in dilute cerine. Examine under a hand lens.

It will be possible to see the vascular bundles, each showing "red" patches of sclerenchyma and xylem towards the outside and inside respectively.

Drawings should show only so much as can be seen accurately.

It will be found in the present instance that although the xylem vessels can be seen, their exact shape is not discernible, nor for that matter is it easy to decide the exact shape of the groups of xylem, though one can just do so in the case of the bundles as a whole.

All that can be determined is:

The shape of the section.

The number, position, relative size and shape of the vascular bundles.

The position of the cortex, pith and medullary rays.

Exercise 36*a*.—Drawing under the Hand Lens

Construct a labelled drawing of the transverse slice of the stem seen under a hand lens, indicating the facts enumerated above only.

Draw to such a scale (which state) that all the above-mentioned parts may be accurately delineated, and label them all (Fig. 15).

Microscope Study.

Until students become expert in section cutting they are advised to aim to cut sections which include samples only of the entire structure and to avoid trying to obtain sections right across the organ.

EXERCISE 37.—CUTTING AND SELECTING A SECTION FOR STUDY UNDER THE MICROSCOPE

Having trimmed off the material at right-angles to its length, lay the razor flooded with 25% spirit on the trimmed surface so that its edge is along a diameter. Cut with a slicing action and aim to remove a section which is a sector and includes one-eighth to one-quarter of the complete transverse section (Fig. 16, p. 56). After cutting the first section rotate the material about one-quarter of a complete turn so that the razor enters it to cut the second section near where it emerged after cutting the first. Repeat the processes and so obtain a number of sections.

If, during sectioning, the end of the material becomes even slightly oblique, trim it off to renew the perfectly transverse surface.

Select a section for (*a*) thinness (Exercise 12*a*, p. 32).
(*b*) truth of direction (Exercise 31, p. 42).
Treat with phloroglucin and hydrochloric acid and mount in dilute glycerine (§ 11, test 14). Examine under the low power.

The Zone Drawing

It will be found that in a satisfactory section, especially at its thin edges where the razor entered or came out of the material, every element may be seen. Many of them, however, are too small for their exact shape to be determined and hence they cannot be drawn. On the other hand, in addition to what was visible under the hand lens one can now see the lines of demarcation between the collenchyma just under the epidermis and the parenchyma within ; the sclerenchyma and the phloem ; the phloem and the cambium ; and the cambium and the xylem.

EXERCISE 37*a*.—DRAWING UNDER THE LOW POWER

Construct a " zone drawing " in which the various groups of tissue are drawn true to shape in outline, but in which no

individual element is shown (Fig. 16). The drawing should be labelled and include sufficient of the section to allow the rest to be imagined, and should be to a stated scale of about

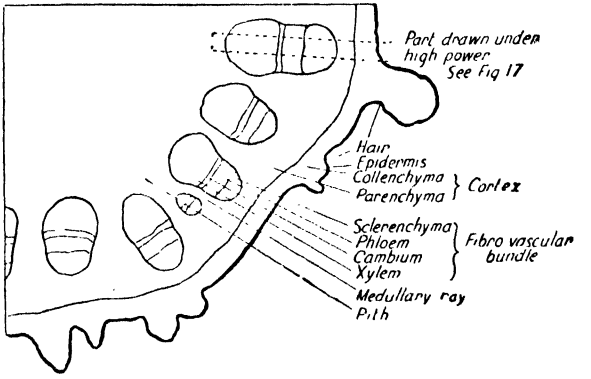

FIG. 16.—Zone Drawing of Transverse Section of Sunflower Stem. × 20 approx.

× 50. When the drawing is completed its area should be indicated in the hand lens drawing (Fig. 15).

High Power Drawing

The hand lens and low power zone drawings show the distribution of the tissues : all that remains to be represented is the shape and relationships of the various elements.

For this purpose it is best to draw a radial strip of the section as seen under the high power. This need be only two or three cells broad, and where any tissue is deep radially, much of it may be omitted if the amount omitted is indicated in the drawing (Fig. 17).

Students usually find great difficulty in deciding on what scale to make their drawings, and having done so in keep-

ing to it. **Drawings cannot be too large** except for convenience; students frequently make them too small for

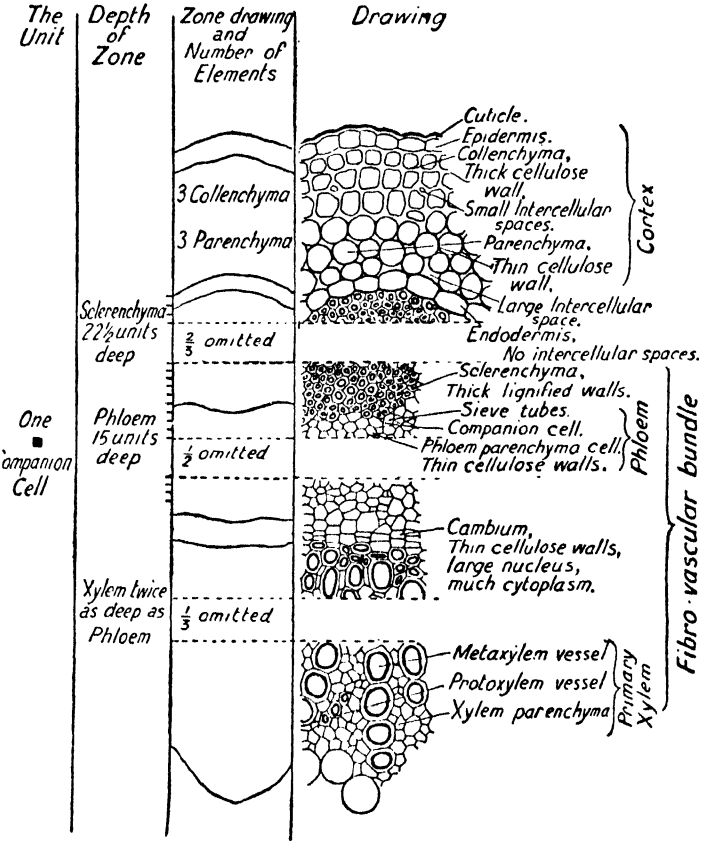

FIG. 17.—Illustrating a method of planning a High Power Drawing. Part of Transverse Section of Sunflower Stem. × 110 approx.

accuracy. They are advised to follow some scheme for determining the scale and maintaining the proportions such as is given in Exercise 37*b*, p. 58.

For the drawing reproduced in Fig. 17 the companion cells were represented with an average diameter of 2 mm.

The complete phloem region in the section had a radial depth equal to 15 companion cells = drawing depth of 30 mm.

The sclerenchyma region was found to be one and a half times as deep as the phloem and the xylem twice as deep, and so on.

To avoid much repetition of drawing and to allow the complete sequence of tissues to be shown in one drawing it was decided to omit :

$\frac{2}{3}$ of the sclerenchyma.
$\frac{1}{2}$ of the phloem.
$\frac{1}{2}$ of the xylem.

In Fig. 17 the sieve tubes are on the average two and a half times as broad as the companion cells. They were drawn approximately 5 mm. in diameter.

Exercise 37*b*.—Drawing under the High Power

Select under the low power the thinnest and clearest region of the section. Indicate this region in the zone drawing. Focus under the high power.

Find the smallest type of element—often the companion cells of the phloem.

The exact shape can be seen and **the scale of the high power drawing must be large** enough to allow accurate representation and full labelling.

Make a trial of the size necessary. Use the actual cell as a unit by which to estimate the extent of the various tissues in the section, and the drawing just made of it as a unit of paper measurement, and by this means plan out in light lines a large zone drawing of the area it is intended to draw under the high power. Then draw in the elements.

To assure that the individual elements are drawn to scale, count the number of elements along a radial line through the zone and divide the corresponding region of the zone drawing similarly. Also check the estimate of element size so made by comparing with the unit (companion cell).

§ 18. Longitudinal Radial Sections

EXERCISE 38.—SUNFLOWER (*Helianthus annuus*) STEM

Preparation of Surface for Cutting

Select a piece of stem one to one and a half inches long, and with a sharp knife or razor cut it lengthways down the middle so that the cut divides two vascular bundles on opposite sides of the stem.

Holding the Material

Grip one piece of the halved stem about midway along its length between the tips of the thumb and middle finger of the left hand, so that the curved face of the material is directed towards the palm of the hand. Support the material in a horizontal position by putting the tips of the index and third fingers under the rounded face (Fig. 18, p. 60).

Cutting

Lay the razor flat on the trimmed face of the material with its edge about 2 mm. from the end nearest the little finger (Fig. 18), and with a slicing action aim to remove a very thin section including all tissues from the surface to the centre of the stem.

After a number of such sections have been removed the vascular bundle will have been cut away from the end few mm. Then commence cutting by laying the razor on

the material with its edge about 4 mm. from the end, and so on.

Selection of Section and Mounting

Select a section for thinness as in Exercise 12*a*, mount it in water and examine under the low power. If it has not been cut approximately longitudinally the lumina of the vessels

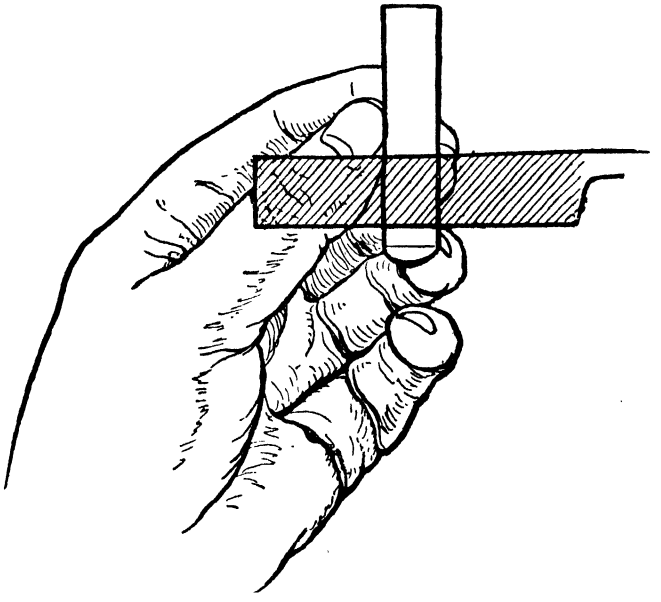

FIG. 18.--Method of holding material for cutting Longitudinal Radial Sections.

will appear as obvious holes in the section. If these do not show, the section is sufficiently truly longitudinal. Treat it with phloroglucin and hydrochloric acid and mount in dilute glycerine. Examine under the low power. A truly radial section will show annular, spiral and pitted vessels in that sequence from the inside outwards.

Interpretation of Longitudinal Sections

Owing to the fact that most of the elements have curved or faceted longitudinal walls, longitudinal sections are not as diagrammatic as transverse ones. Comparison of position of structures seen in the longitudinal section with the same relative position in transverse ones will often assist students in interpreting them.

Drawing

Zone drawings need not be constructed of longitudinal sections unless the distribution of the tissues differs from what would be expected from the appearance of the transverse section. That is not the case in the present instance; hence a high power drawing only is necessary.

Sketch out the zones lightly, determining the proportions in the same way as when drawing the transverse section (Exercise 37*b*, p. 58). Use that drawing to help you. Then draw in the individual elements. Some of each type should be drawn in surface view and others as they appear in optical section, as the section would appear were it thin enough. Label the drawing.

Care should be taken that the ends of elements of each type are included, as these are characteristic in certain instances.

§ 19. Drawing Secondary Tissues

EXERCISE 39.—SUNFLOWER (*Helianthus annuus*) STEM. ORIGIN OF INTERFASCICULAR CAMBIUM

A hand lens drawing need not be constructed, since the difference between this stem and that already drawn in Exercise 36*a*, p. 54, would not be obvious at so small a magnification.

Cut a number of transverse sections. Select one (Exercises 12*a*, p. 32 and 31, p. 42), and mount in magenta and cotton blue in lactophenol (§ 16, *a*, p. 49). Construct a zone drawing to show the position of interfascicular cambium and a high power drawing of part of the cambium only. Label the drawings.

EXERCISE 40.—SUNFLOWER (*Helianthus annuus*) STEM. SECONDARY THICKENING

Cut a moderately thin but complete transverse slice and treat with phloroglucin and hydrochloric acid (§ 11, test 14). Make a hand lens drawing to show the zones of epidermis, cortex, fibres, phloem and xylem.

Cut transverse section, mount in magenta and cotton blue in lactophenol, § 16, *a*, p. 49. Secondary xylem will stain red a little earlier than primary xylem. Make a zone drawing to show the regions and group your labels according to the origin of the tissues from :

Apical meristem.
Fascicular cambium.
Interfascicular cambium.

Make a large scale high power drawing to include samples of all tissues (Exercise 37*b*, p. 58).

When drawing the secondary tissues pursue the following methods (Fig. 19).

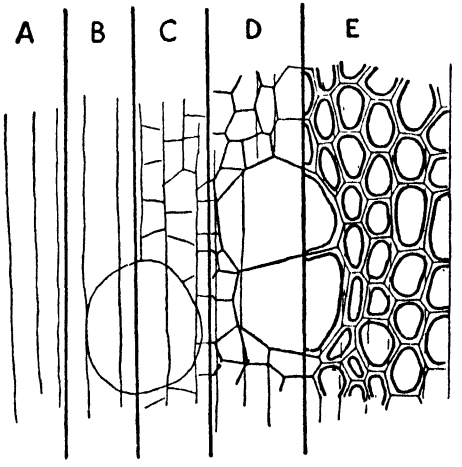

FIG. 19. — Stages in drawing Secondary Xylem. Transverse Section of Sunflower Stem. × 275 approx.

Sketch in, lightly, parallel lines the correct distance apart for the rows of secondary elements (Fig. 19, *a*).

Sketch in, in their correct positions and proportions, the large xylem elements (Fig. 19, *b*).

Draw the tangential walls of the phloem and non-lignified xylem elements and the middle lamellæ of the tangential walls of the lignified elements (Fig. 19, *c*).

Complete the non-lignified elements and the middle lamellæ to the lignified ones (Fig. 19, *d*).

Draw in the inner limits of the lignified walls (Fig. 19, *e*).

§ 20. Structures in Woody Stems

Exercise 41.—Lime (*Tilia*) Stem, 3 to 4 Years Old. Transverse Section

Treat in general as in § 17, p. 53 *et seq.*, and refer to Exercise 40, p. 62, when making the high power drawings. Use the phloroglucin preparation for the hand lens drawing and show in it:

Periderm.
Narrow cortex.
Radiating triangles of secondary phloem (red on account of the sclerenchyma present).
Medullary rays (yellow triangles continued from their apices as lines in the xylem).
Annual rings of wood.
Pith.

When cutting the transverse section be sure to include all tissues from pith to periderm, or if this proves too difficult, make two sections, one of the pith outwards and the other of the periderm inwards, and build up the zone drawing from both.

In the zone drawing differentiate in addition to those features shown in the hand lens drawing:

The medullary sheath and the primary xylem at the border of the pith.
The open texture of the spring wood.
The cambium.
Sclerenchyma groups and sieve tube containing areas in the phloem.
Parenchyma and collenchyma in the cortex.
Phellogen and phellem in the periderm.
Primary and secondary medullary rays.

High Power Drawing :

Use the phloroglucin preparation for the lignified elements and the preparation in magenta and cotton blue in lacto-phenol (§ 16, *a*, p. 49) to study living elements.

For the high power drawing select a strip whose midline is one of the primary medullary rays.

Draw on a large scale four parts only of the strip :

The part including the periderm and cortex.

A small region around the point where a medullary ray crosses the cambium.

A small part where spring and summer wood are in contact.

A part of the pith and primary xylem.

In the xylem region show :

The large vessels. These have bordered pits on the side walls adjoining other vessels or tracheids.

The tracheids with cavities smaller than the vessels and bordered pits on the side walls.

The wood fibres, recognised by their small cavities, simple pits and the absence of contents.

Xylem parenchyma cells which all have a small cavity containing cytoplasm and a nucleus.

The medullary rays, consisting of rows of living oblong cells with half-bordered pits where they abut on a vessel.

In the phloem show :

Lignified bast fibres.

Flattened parenchyma cells, sometimes containing starch or crystals of calcium oxalate.

The large sieve tubes with small companion cells.

The narrow secondary medullary rays, crossing all the zones radially.

Exercise 42.—Lime (*Tilia*) Stem, 3 to 4 Years Old. Longitudinal Radial Section

Treat as in § 18. Ex. 38, p. 49 *et seq.*

The development of the lime stem is often eccentric, so that

the pith is situated a little to one side of the centre of the stem. The arrangement of the tissues is radial around the pith, hence in this case the stem must be split through the centre of the pith (the organic centre) instead of through the true centre.

A zone drawing should be constructed in which the limited depth of the medullary rays is indicated.

Exercise 43.—Lime (*Tilia*) Stem, 3–4 Years Old. Longitudinal Tangential Section

Sections required :

Longitudinal tangential sections are cut longitudinally down the stem and at right-angles to the longitudinal radial section (Fig. 9, p. 41). They are specially useful for studying the " end on " appearance of medullary rays, the edges of radial walls and the surface of tangential walls of the elements. The only parts of these sections that are of value are the middle parts close to line A–B in Fig. 9. On either side of this line the medullary rays, etc., run obliquely through the section and are difficult to see and understand. Hence when it is required to study the tangential appearance of material, sections must be cut at various depths corresponding to the distribution of the tissues.

In this instance the construction of the secondary phloem and xylem are of most importance.

Cutting the Sections

Hold the material which has been used in the last exercise as directed in § 18, p. 59, but with the rounded face uppermost. Trim off just sufficient of the rounded surface to remove the periderm and the cortex. Then cut small sections from the surface of the exposed phloem.

Later trim down to the xylem and again aim for small sections cut at right-angles to the medullary rays.

Mounting

Treat some sections with phloroglucin and hydrochloric acid and mount in dilute glycerine.

Mount others in magenta and cotton blue in lactophenol. The latter will show the sieve plates and the contents of living cells stained blue.

Drawing

Construct two zone drawings (1) of the phloem region and (2) of the xylem region. Make high power drawings of representative parts of both.

EXERCISE 44.—ELDER (*Sambucus nigra*) STEM ONE YEAR OLD. LENTICEL AND PERIDERM

Make a morphological drawing to show the distribution and shape of the lenticels to be seen as brown oval patches in the surface of the stem.

Preparation of Material for Cutting

Select a large lenticel near one end of the piece of stem and trim the material down till the lenticel just shows in the perfectly transverse surface. Cut away, at about 45°, the end of the stem on the opposite side to the lenticel so that only about a quarter of the transverse surface with the lenticel in its middle remains.

Cutting

Hold the material with the oblique surface towards the razor hand. Lay the razor on the transverse surface with the edge about 1 mm. away from the lenticel and aim for a radial strip which includes the lenticel. By cutting soft outer tissues and hard inner ones at one and the same time, splitting of the section is usually avoided.

Mounting

Treat some sections with phloroglucin and hydrochloric acid and mount in dilute glycerine, and mount others in magenta and cotton blue in lactophenol.

Drawing

Make a zone drawing to show the regions: Pith, primary xylem, secondary xylem, cambium, secondary phloem, pericycle, cortex, periderm and lenticel.

Make a high power drawing of the lenticel and part of the neighbouring cork, cork cambium, cortex, etc.

§ 21. Additional Exercises in Stem Structure

EXERCISE 45.—CURLED DOCK (*Rumex crispus*) STEM

Study as in Exercises 36 and 37, p. 53 *et seq.*, and make an additional mount in magenta and cotton blue in lactophenol.

Especially observe the distribution of the sclerenchyma and collenchyma, and the patches of chlorenchyma inside the epidermis.

EXERCISE 46.—WHITE DEADNETTLE (*Lamium album*) STEM

Study as in Exercises 36, 37 and 38, p. 53 *et seq.* Observe the four pillars of collenchyma at the angles; the endodermis with casparian bands; the four open vascular bundles and the hollow pith.

EXERCISE 47.—MARROW (*Cucurbita Pepo*) OR WHITE BRYONY (*Bryonia dioica*) STEM

Study as in Exercises 36, 37 and 38, p. 53 *et seq.* In the zone drawing show:

Epidermis with hairs.
Cortex of collenchyma and parenchyma.
Starch sheath.
Pericycle ring of sclerenchyma.

Open bicollateral vascular bundles with :

External phloem.
Cambium.
Xylem.
Internal phloem.

Pith in which there is often a cavity.

Exercise 48.—Deadly Nightshade (*Atropa Belladona*) Stem

Study as in Exercises 36 and 37 and make an additional mount in magenta and cotton blue in lactophenol. Keep this last and make it permanent (§ 16, *b*, p. 49).

Note especially the small groups of medullary phloem just inside the ring of primary xylem elements which border the pith.

Exercise 49.—Maize (*Zea Mais*) Stem

In monocotyledon stems the vascular bundles are arranged in several concentric circles or scattered. They are closed, i.e. do not possess a cambium, and generally have a sclerenchymatous sheath.

Study as in Exercises 36, 37 and 38, p. 53 *et seq.*, and make additional preparations according to § 16, *a* and *c*, pp. 49 and 50. In the zone drawings show :

Epidermis.

Cortex { Hypoderma of sclerenchyma.
Parenchyma.

Scattered vascular bundles, of which the outer ones are small and have a thick sclerenchyma sheath, the inner ones are large and have a thinner sheath.

Under the high power draw the outer part of a radial strip from the cuticle to the inner limit of one of the outermost vascular bundles, and also one of the inner vascular bundles.

Each bundle is a closed collateral one and contains :

The phloem, consisting of sieve tubes and companion cells.

The xylem, consisting of two large pitted vessels, several spiral and annular protoxylem vessels. The innermost protoxylem vessel becomes torn so that isolated rings lie in a cavity.

The sclerenchyma sheath.

It is advisable that students with limited time at their disposal postpone the remainder of this section till they have studied typical roots and leaves.

Exercise 50.—Lily (*Lilium Martagon*) Stem

Repeat Exercise 49, but note that the sclerenchyma ring is here in the pericycle and has outside it a quantity of chlorenchyma.

Exercise 51.—Creeping Buttercup (*Ranunculus repens*) Stem

Study as in Exercises 36 and 37, p. 53 *et seq.*, and make a double stained permanent preparation according to § 16, *c* and *d*, p. 50.

The vascular bundles are each almost enclosed in a sclerenchymatous sheath and the cambium in the bundles is poorly developed and inactive. The phloem consists entirely of sieve tubes and companion cells.

Thus the bundle strongly resembles in structure a monocotyledon bundle.

Exercise 52.—Japanese Anemone (*Anemone japonica*) Stem

Study as in Exercises 36 and 37, p. 53 *et seq.*, and make a double-stained permanent preparation according to § 16, *c* and *d*, p. 50.

There are several circles of bundles and the bundles have a very poor cambial growth ; thus we have a dicotyledon stem resembling a monocotyledon stem.

EXERCISE 53.—ELM (*Ulmus*) STEM, 1 TO 2 YEARS OLD

Study as in Exercises 41, 42 and 43, p. 63 *et seq.* In the drawings show:

Epidermis with hairs (trichomes) and stomata.

The periderm (if present), consisting of cork and cork cambium formed in the layer under the epidermis.

The cortical parenchyma, amongst which are a few large cells with thickened mucilaginous walls and here and there a crystal sac.

The irregular ring of sclerenchyma (bast fibres).

The phloem zone.

The cambial zone.

The secondary xylem, containing vessels, fibres and parenchyma.

The primary xylem bundles, forming wedges round the pith.

The medullary rays.

The pith or medulla, parenchyma cells with pitted walls and a few large mucilage cells.

EXERCISE 54.—ELDER (*Sambucus nigra*) STEM GATHERED IN EARLY SUMMER

Treat as in Exercise 44, p. 66, and § 16, *c* and *d*, p. 50.

Study the origin of the cork cambium in the hypoderma and of the lenticels inside stomata.

EXERCISE 55.—BLACK CURRANT (*Ribes nigrum*) STEM

Study as in Exercise 44, p. 66, and § 16, *c* and *d*, p. 50, but omit lenticel structure.

Note the deep periderm including cork cambium and phelloderm, also the dead cortical and epidermal cells.

EXERCISE 56.—APPLE (*Pyrus Malus*) STEM GATHERED IN EARLY SUMMER

Study as in Exercise 44, p. 66, and § 16, *c* and *d*, p. 50, but omit lenticel. The cork cambium arises in the epidermis.

EXERCISE 57.—DRAGON TREE (*Dracæna*) STEM. MONOCOTYLEDON WITH SECONDARY THICKENING

Make preparations according to § 11, p. 35, test 14, and § 16, *c* and *d*, p. 50.

In the zone drawing show :

The central primary region with scattered primary bundles which are concentric and closed, the xylem enclosing the phloem. (The ground tissue cells are not in straight rows.)

The zone of secondary tissue in which the ground tissue parenchyma is lignified and in radial rows.

The secondary bundles, like the primary, but elliptical or elongated radially. (Xylem chiefly tracheids.)

The cambium, or meristem zone, with new bundles developing.

The secondary cortex of thin-walled parenchyma cells in radial rows.

The primary cortex with small leaf-trace bundles and crystal sacs. (The cells are not in straight rows)

The periderm, consisting of cork cells in radial rows and the phellogen.

There may be the dead remains of the epidermis.

Under the high power, draw representative parts of a radial strip (see Exercise 37*b*).

EXERCISE 58.—BOG-BEAN (*Menyanthes trifoliata*) STEM. MARSH PLANT

Study as in Exercises 36 and 37, p. 53 *et seq.*, and make preparations according to § 16, *a*, *c* and *d*, pp. 49 and 50. Note :

The large air spaces and leaf-trace bundles in the cortex and the pith.

The large vascular stele enclosed in an endodermis.

The collateral vascular bundles with vestigial cambium, but well-developed xylem and phloem.

Exercise 59.—Rush (*Juncus*) Stem. Marsh Plant

This is another example of a marsh plant. Its stem has a pith of stellate parenchyma and an outer zone of girders.

Study as in Exercise 58, p. 71.

Exercise 60.—Mare's Tail (*Hippuris vulgaris*) Stem. Submerged Water Plant

Treat as in Exercise 58.

When cutting, hold gently so as to avoid crushing the stem and lay the razor on the trimmed surface with its edge nearly touching the stele. Aim to cut the stele; the cortex will look after itself.

In the zone drawing show:

- The epidermis.
- The broad cortex of thin-walled parenchyma with large intercellular spaces.
- The endodermis with thickened walls and lignified bands in the radial walls.
- The central rope-like vascular cylinder of:
  - Xylem vessels enclosing a parenchymatous pith.
  - Sometimes a vestige of cambium.
  - Phloem, which is a zone of small cells just external to the xylem.
  - A pericycle, between endodermis and outermost sieve tubes.

In the high power drawing of the radial strip omit most of the cortex.

Exercise 61*a*.—Water Violet (*Hottonia palustris*) Stem, Submerged Part

Treat as in Exercise 60.

EXERCISE 61*b*.—WATER VIOLET (*Hottonia palustris*) STEM, AERIAL PART

Study as in Exercises 36 and 37, p. 53 *et seq.* Note:

The strong tissue near the surface; the middle is hollow.

The pericyclic zone of sclerenchyma just inside the endodermis.

The ring of separate vascular bundles with xylem vessels.

Other exercises on water stems are given in Exercises 67 and 71, pp. 77, 79.

EXERCISE 62.—DUTCHMAN'S PIPE (*Aristolochia sipho*) STEM, YOUNG. A CLIMBING STEM

Observe inside the endodermis a continuous zone of sclerenchyma constituting the pericycle.

As secondary growth takes place this is burst open and the gaps are at first repaired by the formation of sclerotic cells. The inter-fascicular cambium forms parenchymatous medullary rays only, hence the primary bundles retain their individuality.

In older stems the secondary growth of the vascular bundles crushes the pith and breaks the sclerenchyma ring.

EXERCISE 63.—LILY OF THE VALLEY (*Convallaria majalis*) RHIZOME

Make preparations according to § 11, test 14, and § 16, *a*, *b*, *c* and *d*.

In the drawings indicate:

The thick-walled epidermis.

The broad cortex.

The thickened endodermal cells.

The narrow pericycle.

The ring of collateral vascular bundles.

The inner concentric vascular bundles.

EXERCISE

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This space is left blank for notes on exercises in stem structure other than those set out above.

CHAPTER IV

OVERCOMING DIFFICULTIES WITH SMALL, THIN OR SOFT OBJECTS

§ 22. Bundling

IN cutting thin material it is difficult to maintain the true direction of the sections. For transverse sections of sufficiently firm material, grasping a number of short lengths together in a bundle and cutting them simultaneously often overcomes the difficulty, truth of direction being judged by the appearance of the cut end of the bundle rather than of the individual structure.

Cylindrical material such as roots, stems or cylindrical leaves may with advantage be tied into bundles with a wrapping of cotton.

EXERCISE 64.—BROOM (*Genista præcox*) STEM. ASSIMILATING SWITCH STEM

For cutting, hold a bunch of short lengths of stem together and aim to cut transverse sections of the central ones.

Treat sections according to § 11, test 14, and § 16, *a*.

In the drawings show :

The epidermis with very thick cuticle and with stomata restricted to the grooves between the ridges of the stem.

The cortex, with strands of sclerenchyma beneath the epidermis in the ridges, and assimilatory tissue (chlorenchyma) occupying the sides of the ridges bordering on the grooves.

The hairs in the grooves.

The vascular tissue enclosing a pith and consisting of primary xylem, secondary xylem, cambium and secondary phloem.

EXERCISE 65.—OTHER SWITCH STEMS

(*a*) *Casuarina equisetifolia*, (*b*) Broom (*Cytisus scoparius*), (*c*) Gorse (*Ulex europæus*), (*d*) Whortleberry (*Vaccinium Myrtillus*), (*e*) Rush (*Juncus*).

EXERCISE 66.—FLAG (*Iris*) ROOT. TYPICAL MONOCOTYLEDON ROOT STRUCTURE

Select a number of fairly straight pieces of root. Cut them into lengths of about one inch. Hold them in a bundle and cut transverse sections.

Treat selected sections according to § 11, test 14, and § 16, *a* and *b*.

Construct suitable zone and high power drawings to show :

The piliferous layer with remains of root hairs.

Several outer layers of the cortex thickened and cuticularised (exodermis).

The parenchymatous inner cortex.

The endodermal cells with inner and radial walls strongly thickened and with thin-walled passage cells placed here and there opposite the protoxylem strands.

The pericycle between the protoxylem and endodermis.

The xylem strand with external protoxylem.

The phloem strand.

The ground (conjunctive) tissue consisting of central pith and the cells between the xylem and phloem strands ; this may be more or less thickened and lignified.

§ 23. Hardening in Spirit

When material is so soft that it gives in front of the razor and is difficult to cut for this reason, it should be soaked in strong spirit. The longer it soaks the harder it will become, and after a time sectioning will be possible. Even then both the material and the razor must be kept wet with a strong spirit during the process.

Objects thick enough should be held singly. Thin objects which become sufficiently rigid may be bundled, otherwise they must be embedded in pith or carrot (§ 24, p. 78).

Exercise 67.—Water Stems

The material given below is usually soft. Cut transverse sections of single lengths, bundles or pieces embedded according to the thickness and rigidity of the object.

(*a*) *Myriophyllum :* Central stele as in *Hippuris*, Exercise 60, p. 72, but one circle of large spaces in the cortex. Cells with cluster crystals of calcium oxalate projecting into the spaces.

(*b*) *Elodea :* Resembling *Ceratophyllum* (Exercise 71, p. 79).

(*c*) *Potamogeton natans :* The vascular bundles retain their individuality, but the xylem vessels have disappeared and are represented by spaces ; small bundles of sclerenchyma may be seen in the cortex.

Exercise 68.—Buttercup (*Ranunculus repens*) Root. Typical Dicotyledon Root

This root is often rather soft : harden by soaking in spirit for a time and then cut by the bundling method. Treat selected sections according to § 11, test 14, and § 16, *c* and *d*.

In drawings show :

The piliferous layer with root hairs.
The cortex of parenchyma.
The endodermis with cuticularised radial walls.
The pericycle.
The small number of xylem and phloem strands alternating with one another.
The conjunctive tissue between the strands.

Exercise 69.—Cress Seedlings. Root Hairs

Shave off some of the surface tissue of the root with root hairs attached. Mount in magenta and cotton blue in

lactophenol. Observe that the root hairs are unicellular and consist of a cellulose wall lined with cytoplasm containing a nucleus. There is a central vacuole containing sap.

§ 24. Embedding in Carrot or Pith

Sometimes neither of the methods described in §§ 22 and 23 leads to success. Resort must then be had to embedding. In this method the material is clasped between two pieces of carrot or the two halves of a piece of elder stem pith. The carrot or pith is cut at the same time as the material to which it gives support. Obviously, since the material is hidden in the embedding substance, it is impossible to judge whether truth of cutting direction is being maintained, unless the material is embedded with accurate reference to the lines of the carrot or pith. For transverse sections the material should be embedded perfectly longitudinally, and for longitudinal ones truly transversely in the pith.

Exercise 70.—Pea or Bean Roots, Young. Three Regions of Root Growth

Hold a piece of pith (4–5 cms. long), or a piece of carrot (1 × 1 × 5 cms.), vertical on the bench between the finger and thumb of the left hand. With a sharp razor or scalpel make a vertical cut down the pith or carrot for about half its length, pressing the finger and thumb together while the cut is being made.

Insert, perfectly longitudinally between the two cut faces, a thin paint-brush handle or a matchstick, and squeeze the two parts on to it so as to make grooves down both of the cut surfaces. Into the channel so made insert the root, tip uppermost.

Cut transverse sections of pith and root together. From

amongst the first few sections select a thin one and mount according to § 16, *a*. Examine the apical meristem cells.

Cut other sections and mount selected ones from various levels in the root and so determine the three regions of growth :

The region of cell division just under the root cap where the cells have dense protoplasmic contents and large nuclei.

The region of cell vacuolation just below, where the cells are elongating rapidly, and are seen to have large vacuoles.

The region of differentiation where growth is approaching completion and the cells are taking on their final form as xylem vessels, sieve tubes, etc.

Make a drawing of the characteristic appearance of cells in each of the first two regions, and zone and high power drawings of the completed primary structure as seen in the third.

Exercise 71.—Hornwort (*Ceratophyllum*) Stem. Submerged Water Stem

Embed longitudinally in pith, cut transverse sections and make preparations according to § 16, *a* and *b* only.

In drawings show :

The epidermis without cuticle or stomata.

The cortex of parenchyma with a ring of air spaces and many tannin sacs.

The true endodermis.

The central vascular cylinder with the xylem reduced to a central passage with thickened walls and surrounded by xylem parenchyma : there are no vessels.

The phloem, containing a ring of well-marked sieve tubes.

Exercise 72.—Maize (*Zea Mais*) Radicle. Structure of Root Apex

Cut off the apical 5 mm. of the radicle, harden in spirit, embed horizontally in pith and cut a complete series of longi-

tudinal sections. Look through them all and select the most median section. If it is thick, mount in caustic soda, otherwise in glycerine, and draw to show:

The root cap.

The periblem which gives rise to the cortex.

The plerome which forms the central cylinder.

EXERCISE 73.—BEAN (*Vicia Faba*) ROOT, 6 TO 7 INS. FROM APEX. EARLY SECONDARY THICKENING OF ROOT

Treat as in Exercise 70, p. 78. Make additional preparations according to § 16, *c*. Construct drawings to illustrate:

The origin of the cambium between the xylem and the phloem.

The appearance after some secondary growth has occurred, indicating:

The primary xylem strands in the centre.

The secondary xylem external to the primary xylem.

The cambium region.

The region of secondary phloem.

The position of the primary phloem, which is indicated by the four groups of bast fibres, external to the secondary phloem and alternating with the primary xylem strands.

The cortex.

EXERCISE 74.—PINE (*Pinus sylvestris*) ROOT. WOODY ROOT

The pine root is included here, although not an Angiosperm, on account of the ease with which the primary xylem groups may be recognised.

Cut a transverse section, being sure to include the organic centre of the root where the primary xylem is situated. Treat selected sections according to § 11, test 14, and § 16, *a*.

In the drawings indicate:

The primary xylem with resin ducts nearly encircled by the protoxylem elements.

Secondary xylem with resin ducts.
Cambium.
Secondary phloem.
Medullary rays.
Pericycle with resin ducts.
Periderm.

EXERCISE 75.—ELM (*Ulmus*) ROOT. WOODY ANGIOSPERM ROOT

Treat as in Exercise 74, p. 80, and make suitable drawings.

EXERCISE 76.—CHERRY LAUREL (*Prunus Laurocerasus*) LEAF. TYPICAL BIFACIAL LEAF

From the middle of the leaf cut out about 1·5 cm. of the midrib with about ·5 cm. breadth of lamina on either side of it.

Insert this into the cleft made in a piece of pith (Exercise 70, p. 78) so that the midrib is vertical in it.

Hold the pith for cutting in such a way that the edge of the razor is parallel with the surface of the leaf, so that the razor may have the minimum distance to travel through the material and the section cannot be thin at the side the razor enters and thick at the other. Cut transverse sections of pith and leaf together.

Treat sections selected for thinness, etc., especially of the midrib, according to § 11, test 14, and § 16, *c* and *d*.

Treat sections selected for thinness, etc., especially of the mesophyll, according to § 16, *a*, *b*, *c* and *d*.

Construct a zone drawing to show the regions of the leaf and a high power drawing of a strip through the leaf including one side of the midrib and a contiguous part of the lamina.

In the midrib there is to be seen :

The thick-walled epidermis.
The collenchyma internal to both the upper and lower epidermis.

The crescent of vascular tissue with parts in the following order, beginning from above :

A group of sclerenchyma fibres.
Xylem.
Cambium.
Phloem.
Sclerenchyma.

The bundle sheath consisting of several layers of parenchyma cells containing tannin.

At the side of the midrib the section should show :

The upper epidermis with cuticle but no stomata.

The palisade layer of elongated mesophyll cells, each with many chloroplastids.

The spongy layer of mesophyll with larger intercellular spaces and fewer chloroplastids.

The lower epidermis.

The stomata on lower side only.

Small groups of xylem and phloem may be present in the lamina. They are the veins—vascular bundles cut across in various directions.

EXERCISE *76a*.—CHERRY LAUREL LEAF. LEAF SURFACES

Roll a piece of leaf round a piece of pith and cut very thin surface sections from one face of the leaf. Repeat with the other leaf surface. Mount selected sections in glycerine and make high power drawings.

In the upper surface section there should be visible :

The tabular epidermal cells without chloroplastids and with no stomata among them.

The ends of the palisade cells showing through the epidermis.

The air spaces between the palisade cells.

In the lower surface section one should see :

The epidermal cells with stomata among them.

The cells of the spongy parenchyma with large intercellular spaces between them.

EXERCISE 77.—CHERRY LAUREL PETIOLE

Cut sections from the middle of the petiole and treat according to § 11, test 14, and § 16, *a*.

The construction is very similar to that of the midrib of the leaf.

EXERCISE 78.—HYACINTH (*Hyacinthus orientalis*) LEAF. TYPICAL ISOBILATERAL LEAF

The hyacinth leaf tends to be soft : harden, if necessary, by soaking in strong spirit, and while cutting keep the razor wet with strong spirit. Sections may be obtained by embedding in pith as in Exercise 76, p. 81, but owing to the softness of the material crushing usually occurs. Better results are obtained by a modification of the bundling method.

Take one length of leaf which has been hardened and gently roll it up so that the veins run down the roll. Roll a second leaf round the first and repeat till a convenient sized roll is obtained. Then cut the roll transversely, taking care not to crush it with the left hand.

Mounting to Avoid Crushing

The section is even more delicate than the complete piece of leaf, and the weight of the coverslip may crush it unless precautions are taken. To avoid this lay two coverslips a little less than a coverslip breadth apart on the slide and mount the section between them, bridging the two supporting slips with the actual covering one.

Mount the transverse section of the hyacinth leaf according to § 16, *a* and construct drawings to show :

The epidermis with cuticle and stomata on both surfaces.

A well-marked respiratory cavity under each stoma.

The mesophyll, consisting of about two layers of rounded cells containing chloroplastids and central, thin-walled, rounded parenchyma cells containing few or no chloroplastids. (Irregular cavities may be formed by some of

these cells breaking down. There are raphides in some of the cells.)

The vascular bundles, consisting only of xylem and phloem, the former towards the morphologically upper side of the leaf; here and there small transverse bundles connecting the main parallel longitudinal ones.

Exercise 79.—Hyacinth Leaf, Fresh. Movement of Guard Cells

Cut the surface of the leaf with a scalpel and lift a small tongue of the surface tissue. Bend this back, and then with a quick pull strip off the tongue and immediately immerse the stripping in water. Part of this will appear as a thin translucent skin. This is the epidermis. Cut off part of the epidermis and mount outside uppermost in water.

Draw, under the high power, a stoma with the surrounding epidermal cells.

Remount in 5% salt solution and watch a stoma until the pore is closed and the cytoplasm just commences to shrink away from the walls at the ends of the guard cells.

Draw the stoma in the closed condition.

Rinse off the salt solution and remount in water. Note the reopening of the stomata.

Exercise 80.—Hakea (*Hakea acicularis*) Leaf. Typical Centric Leaf

This is a compound leaf with tough, almost cylindrical segments. It illustrates the centric type of structure.

Make a bundle of a number of leaf branches and cut as described in § 22, p. 75. Treat selected sections according to § 11, test 14, and § 16, *c* and *d*. In zone and high power drawings show :

The epidermis with thick cuticle, and stomata with depressed and over-arched guard cells distributed all round. Each stoma has a large respiratory cavity beneath it.

The well-developed palisade zone, consisting of two layers of cells supported by a series of tubular idioblasts, slightly lignified and extending right to the epidermis.

The central parenchyma, almost destitute of chlorophyll, and with scattered cells containing tannin.

The collateral vascular bundles supported by bundles of sclerenchyma.

EXERCISE 81.—THE RUE (*Ruta graveolens*) LEAF

Treat as in Exercise 76, p. 81. Include in the high power drawing:

The loose texture of the palisade layer.

Groups of the outer palisade cells, each joining on to one inner cell which is often broadened to receive them; this is a collecting cell.

The spherical oil glands just inside the upper and lower epidermis, the cavity bounded by a layer of thin-walled cells surrounded by flattened cells with thicker walls. In the fresh leaf the cavity contains a globule of volatile oil soluble in alcohol. Outside each gland is a group of flattened epidermal cells.

§ 25. Additional Exercises in Root Structure

EXERCISE 82.—PEA ROOTS, GROWN IN SAWDUST AND PRESERVED IN SPIRIT. ROOT BRANCHING

The roots are translucent. Draw under the hand lens to show young roots arising internally.

EXERCISE 83.—SWEET FLAG (*Acorus Calamus*) ROOT. ORIGIN OF ROOT BRANCHES

Treat as in Exercise 71, p. 79, and draw a section which has passed through a young lateral root.

Exercise 84.—Sunflower Radicle, ¼ to ½ in. long. Second Type of Root Apex

Treat as in Exercise 70, p. 78, and draw to show:

The root cap.

The meristem, which gives rise to the root cap and to the piliferous layer.

The periblem, which gives rise to the cortex and endodermis and in a median section is seen to form a single layer of initial cells just at the apex.

The plerome, which gives rise to the central cylinder from the pericycle inwards and forms a group of initial cells at the apex.

Exercise 85.—Pea or Bean Radicle. Further Type of Root Apex

In some dicotyledons with broad root apices the periblem and plerome are not distinct right up to the apex, but there exists a broad transverse zone of meristem under the root cap which gives rise to periblem and plerome lower down.

Treat as in Exercise 70, p. 78.

Exercise 86.—Epiphytic Orchid (*Dendrobium*) Aerial Root

These roots hang freely in the air and absorb rain or dew. The cortical cells contain chloroplastids and the root appears green when wetted.

Treat as in § 11, test 14, and § 16, *a*, *b*, *c* and *d*. Draw to show:

Several layers of non-living cells containing air, whose walls are strengthened by a fibrous thickening. These cells constitute the velamen and occupy the position of the piliferous layer in other roots.

The exodermis, a single layer of cells with thickened radial walls.

The general parenchymatous cortex.
The endodermis with much-thickened walls and passage cells opposite the xylem strands.
The pericycle and conjunctive tissue, often with thickened and lignified walls.
Many alternating xylem and phloem strands embedded in the thick-walled ground tissue.

§ 26. Additional Exercises in Leaf Structure

Xerophytic Structure in Leaves

Exercise 87.—Holly (*Ilex aquifolium*) Leaf

Treat as in Exercise 76, p. 81. Draw to show:
The upper epidermis with no stomata and the outer wall differentiated into three layers, (1) Cuticle, (2) Cuticularised cellulose, (3) Cellulose.
The hypoderma, a single layer of thick-walled supporting cells.
The palisade mesophyll.
The spongy mesophyll, with here and there cells containing crystals of calcium oxalate.
The vascular bundles cut across in various directions, each enclosed in a sheath of parenchyma cells without intercellular spaces; the largest bundles with sclerenchyma, xylem and phloem; the smaller ones with xylem and phloem; and the smallest with one or two tracheids only, enclosed in a sheath.
The lower epidermis with cuticle, small stomata and small respiratory cavities.

Exercise 88.—Rosemary (*Rosmarinus officinalis*) Leaf

The stomata are restricted to grooves in the under surface of the leaf and are further protected by a feltwork of hairs.
Treat as in Exercise 76, p. 81.

Exercise 89.—Oleander (*Nerium oleander*) Leaf

The guard cells of the stomata project from the bottom of pits in the lower surface of the leaf. The pits contain hairs. There are several layers of hypodermal water storage tissue. Note also cluster crystals of calcium oxalate.

Treat as in Exercise 76, p. 81.

Exercise 90.—The India-rubber Plant (*Ficus elastica*) Leaf

The cuticle is thick. There is a three-layered epidermis for water storage, in which special large cells contain cystoliths. The guard cells of stomata are depressed.

Embed pieces of the lamina in pith so that the parallel lateral veins are cut transversely.

Treat as in Exercise 76, p. 81.

Exercise 91.—Crowberry (*Empetrum nigrum*) Leaf

The leaf is permanently rolled. The lower surface lines the cavity so formed and contains the stomata.

Treat as in Exercise 76, p. 81.

Exercise 92.—Marram Grass (*Psamma arundinacea*) Leaf

The leaf is rolled up when dry by the collapse of special thin-walled cells placed at the bottom of longitudinal grooves. The stomata are placed along the sides of the grooves.

Treat as in Exercise 80, and § 16, *a* and *b*.

Exercise 93.—Aloe (*Aloe arborea*) Leaf

The cuticle is thick and the stomata are depressed. The central colourless tissue is for water storage.

Treat as in Exercise 76, p. 81.

Note.—This material seldom stains clearly.

A Floating Aquatic Leaf

Exercise 94.—White Waterlily (*Nymphæa alba*) Leaf

Treat as in Exercise 76, p. 81. Draw to show:

The upper epidermis with cuticle and small stomata.

The thick palisade layer of mesophyll.

The spongy parenchyma with very large intercellular spaces.

The lower epidermis without cuticle or stomata, but with mucilage cells.

The vascular bundles.

The branched internal supporting cells which are thick-walled, partly lignified and show deposits of calcium oxalate.

Two Leaf-like Organs

Exercise 95.—Butcher's Broom (*Ruscus aculeatus*) Phylloclade

Embed in pith. Cut transverse sections and treat sections according to § 11, test 14, and § 16, *a*, *b*, *c* and *d*.

In the drawings show:

Thick-walled epidermis with cuticle and stomata.

The guard cells protected by a plate of cuticle projecting partly over the pore.

The assimilating zone of spongy tissue with chloroplastids.

The large thin-walled, clear, water-storing cells (aqueous tissue) in the centre.

The vascular bundles in a row.

Exercise 96.—Phyllode of *Acacia*

Cut transverse sections in pith and treat sections according to § 11, test 14, and § 16, *c* and *d*.

Exercise 97.—Other Leaf Petioles

Sunflower, Bean, Black currant.

EXERCISE

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EXERCISE

This space is left blank for notes on exercises in root and leaf structure other than those given above.

APPENDIX I

INDEX OF MATERIAL USED AND TABLE OF SOURCES

THE table set out in the following pages lists the material required in the exercises. It may be used for tracing the number of the exercise in which a particular object is cut, but its main end is to supply information as to when and where supplies may be obtained.

In the third column the figures 1 to 12 designate the months of the year, and the actual figures given against a particular plant organ indicate the months which experience has shown are most convenient for collection. In many instances it is possible to obtain what is required during other months, but material is not always in its best condition; gardeners are more ready to cut plants at one time than another, and heavy frosts may preclude digging during part of the winter. Moreover there is more probability of the collector being near some kinds of vegetation at certain times of the year and of recognising wild plants if they are in flower.

The sources from which plants can be obtained are tabulated so that a glance will show what may be obtained from a particular place.

The last two columns refer to Appendix II, in which will be found notes as to the ways in which material must be prepared for use and subsequently preserved till it is required.

Material.	Exercise Number.	Months for Collecting.	SOURCES OF									
			Wholesale Chemist.	Grocer.	Greengrocer.	Fruiterer.	Florist.	Seedsman.	Orchid Specialist or	Horticulturist.	Botanic Garden.	Aquarist or Pond.
Acacia, phylloclade	96	6–9	—	—	—	—	—	—	—	—	+	—
Acorus Calamus, root	83	6–7	—	—	—	—	—	—	—	—	—	—
Almond	20	1–12	—	+	—	—	—	—	—	—	—	—
Aloe arborea, leaf	93	1–12	—	—	—	—	—	—	—	—	+	—
Anemone japonica, stem	52	6–9	—	—	—	—	—	—	—	—	—	—
Apple, fruit	15	1–12	—	—	—	+	—	—	—	—	—	—
,, , stem, see Pyrus.												
Aristolochia sipho, stem, young	62	7–9	—	—	—	—	—	—	—	+	+	—
Artichoke, tuber, fresh	17	10–3	—	—	+	—	—	—	—	—	—	—
,, ,, , preserved	18	10–3	—	—	+	—	—	—	—	—	—	—
Atropa Belladonna, root	27	6–8	—	—	—	—	—	—	—	—	+	—
,, ,, , stem	48	6–8	—	—	—	—	—	—	—	—	+	—
Bean, seed	19	1–12	—	—	—	—	—	+	—	—	—	—
,, , root, older	73	1–12	—	—	—	—	—	—	—	—	—	—
,, ,, , young	71	1–12	—	—	—	—	—	—	—	—	—	—
,, , petiole	97	6–8	—	—	—	—	—	—	—	—	—	—
Belladonna, see *Atropa*.												
Black currant, see *Ribes*.												
Bog-bean, Buck-bean, see *Menyanthes*.												
Brazil nut	22		—	—	—	+	—	—	—	—	—	—
Broom, see *Cytisus* and *Genista*.												
,, , Butcher's, see *Ruscus*.												
Bryonia dioica, stem	47	5–9	—	—	—	—	—	—	—	—	—	—
Buttercup, see *Ranunculus*.												
Castor oil seed	21	1–12	—	—	—	—	—	+	—	—	—	—
Casuarina equisetifolia, stem	65	5–7	—	—	—	—	—	—	—	—	+	—
Ceratophyllum, stem	71	6–7	—	—	—	—	—	—	—	—	—	+
Cherry laurel, see *Prunus*.												
Convallaria majalis, rhizome	63	5–8	—	—	—	—	—	—	—	—	—	—
Cork, bottle	35	1–12	—	—	—	—	—	—	—	—	—	—
Cress seedlings	69	1–12	—	—	—	—	—	—	—	—	—	—
Crowberry, see *Empetrum*.												
Cucurbita Pepo, stem	32 34 47	6–8	—	—	—	—	—	—	—	—	—	—
Cytisus scoparius, stem	65	5–7	—	—	—	—	—	—	—	—	—	—
Deadly nightshade, see *Atropa*.												
Deadnettle, see *Lamium*.												
Dendrobium, aerial root	86	7–9	—	—	—	—	—	—	+	—	+	—
Dock, see *Rumex*.												
Dracaena, stem	57	7–9	—	—	—	—	—	—	—	—	+	—
Dragon tree, see *Dracaena*.												
Dutchman's pipe, see *Aristolochia*.												

IATERIAL.																Method of Preparation and Preservation.	
Bogs and Pond Sides.	Wet Places.	Ditches.	Hedgerows.	Roadsides and/or Waste Places.	Fields.	Meadows.	Sand Dunes.	Heaths.	Woods.	Old Plantations.	Orchards.	Kitchen Garden.	Garden.	Grow from Seed.	Household Vacuum.	Preparation (Letters refer to Appendix II).	Preservation (Numerals refer to Appendix II).
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—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	+	—	—	—	—	—	—	+	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	5
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	C I	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4

Material.	Exercise Number.	Months for Collecting.	SOURCES OF									
			Wholesale Chemist.	Grocer.	Greengrocer.	Fruiterer.	Florist.	Seedsman.	Orchid Specialist or	Horticulturist.	Botanic Garden.	Aquarist or Pond.
Elder, see *Sambucus*.												
Elm, see *Ulmus*.												
Elodea, leaf	8	4–10	—	—	—	—	—	—	—	—	—	+
,, , stem	67	4–10	—	—	—	—	—	—	—	—	—	+
Empetrum nigrum, leaf	91	4–8	—	—	—	—	—	—	—	—	—	—
Ficus elastica, leaf	90	7–9	—	—	—	—	—	—	—	—	+	—
Flag, see Iris.												
,, , Sweet, see *Acorus*.												
Genista praecox, stem	64	6–9	—	—	—	—	—	—	—	—	—	—
Gorse, see *Ulex*.												
Hakea acicularis, leaf	80	6–9	—	—	—	—	—	—	—	—	+	—
Helianthus annuus, fruit	23	1–12	—	—	—	—	—	+	—	—	—	—
,, ,, , petiole	97	6–9	—	—	—	—	—	—	—	—	—	—
,, ,, , radicle	84	1–12	—	—	—	—	—	—	—	—	—	—
,, ,, , stem, with interfascicular cambium	39	6–7	—	—	—	—	—	—	—	—	—	—
,, ,, , stem, primary structure.	36 37 38	7–8	—	—	—	—	—	—	—	—	—	—
,, ,, , stem, secondary structure.	40	9–10	—	—	—	—	—	—	—	—	—	—
Hippuris vulgaris, stem	60	6–9	—	—	—	—	—	—	—	—	—	+
Holly, see *Ilex*.												
Hornwort, see *Ceratophyllum*.												
Hottonia, stem, submerged	61*a*	5–8	—	—	—	—	—	—	—	—	—	+
,, ,, , aerial	61*b*	5–8	—	—	—	—	—	—	—	—	—	+
Hyacinth, bulb	26	9–12	—	—	—	—	—	+	—	+	—	—
,, , leaf, preserved	26 78	12–3	—	—	—	—	+	—	—	—	—	—
,, , ,, , fresh	79	12–3	—	—	—	—	+	—	—	—	—	—
Ilex aquifolium, leaf	87	1–12	—	—	—	—	—	—	—	—	—	—
India-rubber plant, see *Ficus*.												
Iris, rhizome	13	11–3	—	—	—	—	—	—	—	—	—	—
,, , root	66	11–3	—	—	—	—	—	—	—	—	—	—
Juncus, stem	59 65	6–9	—	—	—	—	—	—	—	—	—	—
Kleinia hastata, stem	28	6–8	—	—	—	—	—	—	—	—	+	—

MATERIAL.																		Method of Preparation and Preservation.	
Bogs and Pond Sides.	Wet Places.	Ditches.	Hedgerows.	Roadsides and/or Waste Places.	Fields.	Meadows.	Sand Dunes.	Heaths.	Woods.	Old Plantations.	Orchards.	Kitchen Garden.	Garden.	Grow from Seed.	Household Vacuum.	Preparation (Letters refer to Appendix II).	Preservation (Numerals refer to Appendix II).
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	2
–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	G	5
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	A	3
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	C I	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	F G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	F G	4
–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	F G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	5
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	5
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	A	3
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	5
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	A	1
–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	I	4
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	4

Material.	Exercise Number.	Months for Collecting.	SOURCES OF									
			Wholesale Chemist.	Grocer.	Greengrocer.	Fruiterer.	Florist.	Seedsman.	Orchid Specialist or	Horticulturist.	Botanic Garden.	Aquarist or Pond.
Lamium album, stem	46	3–9	—	—	—	—	—	—	—	—	—	—
Lilium Martagon, stem	50	7–8	—	—	—	—	—	—	—	—	—	—
Lily of the valley, see *Convallaria*.												
Lily, water, see *Nymphaea*.												
Lily, see *Lilium*.												
Lime, see *Tilia*.												
Lupinus hirsutus, seed	24	1–12	—	—	—	—	—	+	—	—	—	—
Maize, see *Zea*.												
Mare's tail, see *Hippuris*.												
Marram grass, see *Psamma*.												
Marrow, see *Cucurbita*.												
Menyanthes, stem	58	5–9	—	—	—	—	—	—	—	—	—	+
Myriophyllum, stem	67	5–8	—	—	—	—	—	—	—	—	—	+
Nerium oleander, leaf	89	1–12	—	—	—	—	—	—	—	+	+	—
Nymphaea alba, leaf	94	6–9	—	—	—	—	—	—	—	—	—	+
Oleander, see *Nerium*.												
Onion bulb	7	1–12	—	—	+	—	—	—	—	—	—	—
Orchid, see *Dendrobium* or *Phajus*.												
Parsnip	16	10–3	—	—	+	—	—	—	—	—	—	—
Pea, radicle	85	1–12	—	—	—	—	—	—	—	—	—	—
,, , root, young	70	1–12	—	—	—	—	—	—	—	—	—	—
,, , ,, , long	82	1–12	—	—	—	—	—	—	—	—	—	—
,, , seed	19	1–12	—	—	—	—	—	+	—	—	—	—
Pellionia pulchra, stem	14		—	—	—	—	—	—	—	+	+	—
Phajus grandiflora, tuber	13		—	—	—	—	—	—	+	—	—	—
Pinus, root	74	9–11	—	—	—	—	—	—	—	—	—	—
Potato tuber	12	1–12	—	—	+	—	—	—	—	—	—	—
,, ,, , young	13	6–7	—	—	—	—	—	—	—	—	—	—
Potomogeton natans, stem	67	5–8	—	—	—	—	—	—	—	—	—	+
Prunus Laurocerasus, leaf	76	6–9	—	—	—	—	—	—	—	—	—	—
,, ,, , petiole	77	6–9	—	—	—	—	—	—	—	—	—	—
Psamma arundinacea, leaf	92	6–9	—	—	—	—	—	—	—	—	—	—
Pyrus Malus, fruit	15	1–12	—	—	—	+	—	—	—	—	—	—
,, ,, , stem, gathered early summer	56	5–6	—	—	—	—	—	—	—	—	—	—
Ranunculus repens, stem	51	5–9	—	—	—	—	—	—	—	—	—	—
,, ,, , root	68	5–9	—	—	—	—	—	—	—	—	—	—

MATERIAL.																Method of Preparation and Preservation.	
Bogs and Pond Sides.	Wet Places.	Ditches.	Hedgerows.	Roadsides and/or Waste Places.	Fields.	Meadows.	Sand Dunes.	Heaths.	Woods.	Old Plantations.	Orchards.	Kitchen Garden.	Garden.	Grow from Seed.	Household Vacuum.	Preparation (Letters refer to Appendix II).	Preservation (Numerals refer to Appendix II).
—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	5
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	5
—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	A	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	C I	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	C I	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	C G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	A	1
																or I	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	I	4
—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	1
—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	—	C I	4
—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	1
—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	F G	4
—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	G	4

Material.	Exercise Number.	Months for Collecting.	SOURCES OF									
			Wholesale Chemist.	Grocer.	Greengrocer.	Fruiterer.	Florist.	Seedsman.	Orchid Specialist or	Horticulturist.	Botanic Garden.	Aquarist or Pond.
Ribes nigrum, stem	55	8–9	—	—	—	—	—	—	—	—	—	—
,, ,, , petiole	97	6–9	—	—	—	—	—	—	—	—	—	—
Rose fruit	10	9–10	—	—	—	—	—	—	—	—	—	—
Rosemarinus officinalis, leaf	88	6–9	—	—	—	—	—	—	—	—	—	—
Rue, see *Ruta*.												
Rumex crispus, stem	45	6–8	—	—	—	—	—	—	—	—	—	—
Ruscus aculeatus, phylloclade	95	6–9	—	—	—	—	—	—	—	+	—	—
Rush, see *Juncus*.												
Ruta graveolens, leaf	81	6–9	—	—	—	—	—	—	—	—	+	—
Sambucus nigra, stem, very young	30	4–5	—	—	—	—	—	—	—	—	—	—
,, ,, , early summer	54	5–6	—	—	—	—	—	—	—	—	—	—
,, ,, , end of first year	44	9–10	—	—	—	—	—	—	—	—	—	—
Sedum spectabile, stem	29	7–9	—	—	—	—	—	—	—	—	—	—
	31											
Spirogyra	9	4–5 9–10	—	—	—	—	—	—	—	—	—	+
Starch, oatmeal	5	1–12	+	—	—	—	—	—	—	—	—	—
,, , pea	4	1–12	+	—	—	—	—	—	—	—	—	—
,, , potato	1	1–12	+	—	—	—	—	—	—	—	—	—
,, , wheat	3	1–12	+	—	—	—	—	—	—	—	—	—
Sunflower, see *Helianthus*.												
Sweepings, floor	6	1–12	—	—	—	—	—	—	—	—	—	—
Tetragonia crystallina, stem	25	7–9	—	—	—	—	—	—	—	—	+	—
Tilia, stem, 3 to 4 years old	28	9–11	—	—	—	—	—	—	—	—	—	—
	41											
	42											
	43											
Ulex europaeus, stem	65	2–8	—	—	—	—	—	—	—	—	—	—
Ulmus, stem, 1 to 2 years old	53	9–11	—	—	—	—	—	—	—	—	—	—
,, , root	75	9–11	—	—	—	—	—	—	—	—	—	—
Vaccinium myrtillus, stem	65	4–8	—	—	—	—	—	—	—	—	—	—
Violet, water, see *Hottonia*.												
Water lily, see *Nymphaea*.												
Whortleberry, see *Vaccinium*.												
Zea Mais, stem	49	7–8	—	—	—	—	—	—	—	—	—	—
,, , radicle	72	1–12	—	—	—	—	—	—	—	—	—	—

MATERIAL.																Method of Preparation and Preservation.	
Bogs and Pond Sides.	Wet Places.	Ditches.	Hedgerows.	Roadsides and/or Waste Places.	Fields.	Meadows.	Sand Dunes.	Heaths.	Woods.	Old Plantations.	Orchards.	Kitchen Garden.	Garden.	Grow from Seed.	Household Vacuum.	Preparation (Letters refer to Appendix II).	Preservation (Numerals refer to Appendix II).
—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	F G	4
—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	G	4
—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	A	1
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	+	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	F G	4
—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	F G	4
—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	F G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	2
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	A	3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	—	G	6
—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	G	6
—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	G	6
—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	G	4
—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	C I	4

APPENDIX II

METHODS OF PREPARATION AND PRESERVATION OF MATERIAL

LETTERS and figures in heavy black type refer to the last two columns in Appendix I.

A. Objects which are used in the exercises in the fresh or dry condition need no preparation other than what is part of the actual examination.

B. Some seeds show their cell contents best if they are soaked before being preserved. Peas should be soaked in water for 24 hours before being fixed by the chromo-acetic method (see **I** below).

C. Radicles of seedlings and young roots may be obtained all the year round by germinating the seeds. For this purpose shallow earthenware saucers or boxes are best. They are filled with sawdust, washed sand or Sphagnum moss, and the previously soaked seeds are set so that the radicle points downwards. The seeds are kept just moist and warm. A few days suffices for the emergence of the radicles, which are allowed to grow to the required length and are then removed and preserved.

D. Young potatoes may be dug early in the season or they may be obtained by keeping a mature potato in a dark and not too dry place. The mature tuber will shoot and, after a time, small tubers will be formed at the expense of the mature one.

E. Maize plants may be grown readily from seed sown in the garden in the spring. It will be found that the common maize grains, such as are usually seen in chicken food, yield the hardiest and most successful plants.

Much of the material used is derived from commonly grown plants, and where it is the intention to collect from the garden

it is advisable to make sure that more than is required for ordinary garden use is planted. Whether plants are collected from a garden close to the laboratory or have to come some distance, it is absolutely essential to ensure that they suffer the minimum drying before they are preserved.

F. In some instances it is important that an experienced botanist should select the part of the plant that is to be cut by the novice. This is true in relation to the sunflower stem. During the early summer the plant makes rapid growth and the stem commences to undergo secondary thickening even before the primary structure is fully completed. To the novice this condition is confusing. Later, when the rate of growth slows down, however, it is possible, by making trial sections, to select lengths in which the primary structure is fully completed, but in which the interfascicular cambium has not yet commenced to form. Later still much of the stem will have produced secondary growth. Similar precautions apply to the apple and elder stem that are used to show the origin of the cork cambium.

G. Most material is examined in the preserved condition and prior to preservation must be killed, fixed so that the cells and their contents retain as nearly as possible the condition in which they were during life, and hardened so that subsequent treatment may not alter their appearance. Hardening is also necessary so that objects that were soft may offer sufficient resistance to the razor. These three processes may usually be satisfactorily accomplished in anatomical material by soaking in alcohol, which confers in addition certain other advantages. It displaces the air which is always present in the intercellular spaces of living material and so leads to sections that are not opaque by reason of air present in them. It also removes water and so paves the way for dehydration which is necessary before sections can be cleared in oily liquids such as xylol or oil of cloves.

The objects to be fixed are cut up into pieces of suitable size, about 1 inch long in the case of stems. The pieces are

then put into a jar and covered with industrial spirit.* The jar is tightly closed with a bung through which the suction pipe of a filter pump projects, and by the action of the filter pump air is drawn out of the vessel. By this means the air in the intercellular spaces is rapidly extracted to allow the fixative to enter, and in due course the material sinks. When this has occurred the pump may be disconnected. The material is left in the industrial spirit for the remainder of 24 hours and is then transferred to 75% spirit for storage.*

I. For cells whose contents are to be examined, other fixing fluids usually yield better results. One of these is chromo-acetic acid.* Small objects, such as root tips, are cut off and dropped immediately into the fixing fluid ; larger ones, such as young potatoes, should be cut up into small pieces first, and seeds such as peas, after having been soaked, should be stripped of their seed coats and split in halves before being immersed in the fluid. The vessel containing the fixing agent and the material is attached to the filter pump and air is withdrawn as in the alcohol method. The objects are left in the fixing agent for 24 hours after they have sunk and are then thoroughly washed with water to remove the fixing agent. The best way to do this is to soak the material in warm water, changed every half-hour for, say, 12 hours. Subsequently, the water must be removed and the material hardened. This process must be gradual or shrinkage will result. Thus after washing is completed the material is transferred to 10% spirit for half an hour, then to 20% spirit for 1 hour, and then through successive grades of alcohol in a similar way up to 95%, in which it is left for 12 hours. It may then be returned to 75% spirit for storage.

1. Some of the objects to be examined must be obtained fresh every time they are required for use. Such material as

* Fixing fluids must always be used in excess. The material to be fixed has a considerable effect on the fluid, and unless there is plenty of the latter it cannot act satisfactorily. Storage fluids need be present in quantities only sufficient to cover the material.

rose fruits show the facts for which they are examined only if they have been recently collected from the bush. If they are kept, they wither and the cell contents disintegrate ; moreover they do not lend themselves to methods of preservation which will retain the characteristic parts.

2. There are a few objects, such as *Spirogyra* and *Elodea* leaves, which must for the best results be examined in the fresh condition, but which may be kept healthy in jars or tanks of water in the laboratory for some months. Precautions should be taken that the water is kept moderately fresh and is not exposed to direct sunlight.

3. The material that is the least trouble is that such as starches and some seeds which, after being purchased, are simply stored in bottles or boxes in the condition in which they are obtained.

4. Most stems and leaves are best stored in 75% spirit in airtight containers. It is very important that the stoppers of the vessels should be effective, or during a period of weeks evaporation will reduce the concentration of the spirit considerably and the material may go mouldy.

5. Certain stems and leaves, such as *Elodea* stem and *Hyacinth* leaves, are best preserved in strong spirit since they require much hardening. On the other hand, soaking for too long in this liquid will make them brittle.

6. Others are too hard for cutting even when they are collected. They should be stored in equal parts of spirit and glycerine.

APPENDIX III

STAINS, REAGENTS, ETC.

The reagents marked * are frequently needed, the others should be available in the laboratory.

IT is advisable that students test the effect of reagents and stains on known material such as is given in No. 12 prior to applying the tests to unknown material.

ACETIC ACID, glacial, is used as a mounting medium to bring out the parts of compound aleurone grains.

ACETIC ACID, dilute.

Glacial acetic acid	1 c.c.
Distilled water	99 c.c.

Dissolves calcium carbonate with the evolution of bubbles of carbon dioxide.

*ALCOHOL, dilute spirit. A 25% solution of industrial spirit in water may be conveniently kept in wash bottles of about 500 c.c. capacity. It is used for flooding the razor and the material during section cutting, for the immersion of the material while it is not actually being cut, and for the immersion of sections while they are being selected.

ALCOHOL, 75% spirit. A 75% solution of industrial spirit in water is used for preservation of material which is to be cut. Stronger concentrations may be advantageously used for material that tends to be soft. Glycerine may be added when material is hard or becomes too brittle.

*ALCOHOL, industrial spirit, 64° O.P., 90% spirit. Industrial spirit is a satisfactory fixative for most plant organs that are to be examined anatomically. It may be used to precede absolute alcohol in the process of dehydration for the

economy of the latter fluid. It precipitates sugars and inulin, coagulates proteins and dissolves chlorophyll, other colouring matters, resins and some fixed oils.

*Alcohol, absolute, must be used as a final dehydrating agent prior to transferring sections to xylol or oil of cloves. Its capacities for dissolving and precipitating are much the same as those of industrial spirit.

Alkanet. A solution of commercial alkannin in absolute alcohol is prepared, diluted with an equal volume of distilled water and filtered.

The solution gives a pink to red colour with oils, resin and caoutchouc, but the action is often very slow.

Ammonia, strong, following nitric acid and warmth, gives a yellow colour (Xanthoproteic reaction) with protein.

Ammonia, 10%, mixed with iodine solution gives a bright red colour with tannin.

*Canada Balsam.

Dissolve 25 grms. of . . Canada balsam in
25 c.c. . . . Pure xylol.

Filter and allow the filtrate to evaporate to the consistency of glycerine.

Is used as a mounting medium for permanent preparations. Objects so mounted must be previously dehydrated with alcohol and cleared in xylol or clove oil.

Chlor-Zinc-Iodine (Schultze's Reagent).

Dissolve 100 grms. . Zinc in
300 c.c. . Pure hydrochloric acid and evaporate the solution to 150 c.c. (sp. gr. 1·8).
Dissolve 12 grms. . Potassium iodide in as little water as possible and
add 0·15 grm. . Sublimed iodine crystals.
Mix the two solutions.

This reagent causes starch grains to swell and turn blue, cellulose walls to turn blue to violet, lignified walls, suberised walls, protoplasm and protein to turn yellow to brown.

CHROMIC ACID is a constituent of many fixing reagents, e.g. chromo-acetic acid.

CHROMO-ACETIC ACID.

Dissolve	0·3 grm.	Chromic acid in
	100 c.c.	Distilled water and
add	0·7 c.c.	Acetic acid.

This is a suitable fixative for the material needed for Exercises 12, 13, 71, etc.

CLOVE OIL is used as a clearing agent after dehydration with absolute alcohol. It is also a solvent for some stains, e.g. light green. When it is used as a solvent it serves two purposes simultaneously.

COPPER SULPHATE is used in the preparation of Fehling's solution.

COTTON BLUE stains protein, protoplasm and mucilage blue. See Stains in lactophenol, below.

ETHER is a solvent for fat and oil.

FEHLING'S SOLUTION.

Solution A

Dissolve	69·28 grms.	Copper sulphate in
	1,000 c.c.	Distilled water.

Solution B

Dissolve	346 grms.	Rochelle salt (Sodium potassium tartrate) and
	130 grms.	Caustic soda in
	1,000 c.c.	Distilled water.

Keep the two solutions separately and just prior to use mix equal parts of A and B.

The mixture when heated with glucose, fructose or maltose gives a yellow to red or brown precipitate.

FERRIC CHLORIDE, neutral solution, produces a blue-black to green colour with tannin.

GLYCERINE is a valuable temporary mounting medium because it has a high refractive index and does not evaporate. It may be used pure for this purpose. It is added to alcohol in equal proportions for the preservation of material that is hard or tends to become brittle. It is a constituent of lacto-phenol.

*GLYCERINE, dilute.

Glycerine.	50 c.c.
Water	50 c.c.

Dilute glycerine is used more frequently than pure glycerine as a temporary mounting medium since it is less treacly to handle.

*HYDROCHLORIC ACID, strong, is used in conjunction with an alcoholic solution of phloroglucin as a test for lignification.

HYDROCHLORIC ACID, dilute.

Pure hydrochloric acid . . .	10 c.c.
Water	80 c.c.

Dilute hydrochloric acid may be used with heat to invert cane sugar into a mixture of glucose and sucrose.

*IODINE.

Dissolve 1 grm. .	Potassium iodide in
100 c.c. .	Distilled water and
add 0·3 grm. .	Sublimed iodine.

Shake at intervals till the latter is dissolved.

Iodine gives a blue to black colour with starch, a brown colour with protein, protoplasmic structures, cuticularised walls and lignified walls, and a faint yellow with cellulose walls. In combination with sulphuric acid a blue colour is produced in cellulose walls which at the same time swell.

LACTOPHENOL.

Mix	100 c.c.	Lactic acid.
	200 c.c.	Pure glycerine.
	100 c.c.	Distilled water.
Add	100 grms.	Phenol crystals and allow to dissolve.

Lactophenol is a temporary mounting medium of high refractive index in which many stains are soluble. It may often be used in place of glycerine.

*LIGHT GREEN. A saturated solution in oil of cloves is used for staining cellulose walls. As a counter-stain to safranin it is valuable.

MAGENTA is used to stain lignified walls in the mixture of stains (q.v.) in lactophenol.

MILLON'S REAGENT.

Stand a small beaker containing	1 c.c.	Mercury
in a fume cupboard and add	9 c.c.	Conc. nitric acid.
Close the fume cupboard and when the action is complete add	10 c.c.	Distilled water.

When proteins are warmed in this reagent they turn brick red.

NITRIC ACID, strong, colours cuticularised walls and proteins yellow, and causes cellulose walls and lignified walls to swell. It is used with ammonia (q.v.) in the Xanthoproteic test for protein.

PHENOL (CARBOLIC ACID) is a constituent of lactophenol, q.v.

*PHLOROGLUCIN. A strong solution of the solid in industrial spirit is used with strong hydrochloric acid as a test for lignification.

*SAFRANIN.

Dissolve	1 grm. of	Safranin in
	100 c.c. of	95% spirit and
add	50 c.c. of	Distilled water.

Safranin is a useful stain for lignified walls. It also stains nuclei.

SCHULTZE'S REAGENT, see Chlor-zinc-iodine.

SODA, caustic. A 5% solution in water is commonly used. It causes iodide of starch to swell and lose its colour, saponifies fats and gives a reddish colour to cells containing tannin.

SODIUM CHLORIDE. A 5% solution.

*STAINS in *lactophenol* (magenta and cotton blue).

Stock solutions of stains.

1% cotton blue in lactophenol.

1% magenta in lactophenol.

Mixture for use.

1% cotton blue in lactophenol . .	4 c.c.
1% magenta in lactophenol . .	2 c.c.
Lactophenol	50 c.c.

SUDAN III.

Make a saturated solution of the solid in 70% absolute alcohol. After some days filter.

Cuticularised walls, suberised walls, fat and oil, when mounted in the stain, gradually pick up the colour : the longer they are left in it the deeper their colour becomes.

SULPHURIC ACID, concentrated, dissolves cellulose and starch.

*SULPHURIC ACID, 75%, causes the conversion of calcium oxalate into calcium sulphate, the change of crystal shape indicating that the process is going on. It also gives a blue colour with cellulose walls previously treated with iodine.

*XYLOL is a solvent for canada balsam, and is also used as a clearing agent.

APPENDIX IV

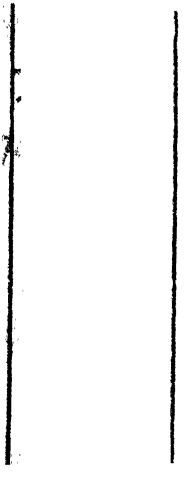
STUDENTS' REQUIREMENTS

BOTANICAL razor.
Scalpel.
Pair of forceps.
Camel-hair brush
Pocket lens.
Duster.
Microscope slides.
Coverslips.
Notebook, containing plain pages of large area with a hard surface, and an HB pencil.
Microscope.
Light source.
Small white porcelain dish.
Wash bottle of 25% spirit.
Water supply above a sink or a wash bottle of water and a vessel for used liquids.
Rack of stains and reagents marked * in Appendix III.

The other reagents listed in Appendix III should be available in the laboratory.

INDEX

Figures are page numbers



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